LINKING PLANT AND SOIL NUTRIENT DYNAMICS IN TEMPERATE AND TROPICAL MONTANE FORESTS

by

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TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Nutrient cycling within watersheds is controlled by feedbacks between plant nutrient use and soil nutrient availability (Chapin III et al. 2011). Nutrients enter ecosystems through weathering of parent material (Walker and Syers 1976; Houlton et al. 2018), biological fixation of atmospheric nitrogen (N) (Houlton et al. 2008), and deposition from rainfall and dust (Chadwick et al. 1999). Once nutrients enter a system, a large portion cycle internally. Some nutrients, like phosphorus (P), can be tightly bound to soil particles, and others, such as organic and inorganic N, can be immobilized and metabolized by microbes in the soil or taken up by plants. Nutrients cycle back into the soil through leaf abscission, and the litter is broken into its original components through microbial decomposition, where it is again available for microbial or plant uptake. As internal ecosystem cycling occurs, nutrients also exit the system through leaching and erosion by wind and water, or through gaseous emissions. Nutrient cycling is not a globally uniform process, however. Climate strongly controls the rate at which internal nutrient cycling occurs, and nutrient cycling rates are much faster in wet and warm than in cool and dry ecosystems. Environmental constraints, including variability in nutrient cycling, influence plants' capacity to grow.

Nutrients limit plant productivity to some degree in all ecosystems (Vitousek and Howarth 1991). However, plants acclimate to this limitation by gaining mycorrhizal symbionts, growing longer roots, altering the physiological mechanism of nutrient

uptake, or storing nutrients (Chapin et al. 2011). Most of our knowledge of long-lived tree nutrition is based upon seedling trees grown under artificial conditions, upon variation in soil nutrient pools that are then assumed to correlate with plant nutrient uptake, or upon changes in foliar nutrient concentrations that are assumed to reflect nutrient uptake rates on an annual basis. However, plant nutrition in long-lived evergreens may differ from our general understanding of plan nutrition that was developed by testing seedlings grown in greenhouses. For example, some evidence suggests that evergreens growing in low nutrient ecosystems are not nitrogen (N) limited because they have low photosynthetic rates per unit of leaf N (PNUE) (Field and Mooney 1986; Reich et al. 1998; Warren and Adams 2004). Evergreens potentially alter their physiology and store N in excess (Warren and Adams 2004), and storage could permit evergreen growth to be independent of N uptake and availability (Proe and Millard 1994). However, little research has directly examined when evergreens take in the bulk of their N in natural systems, so patterns of evergreen N uptake in conjunction with seasonal soil nutrient availability are virtually unknown (though see Chapin and Kedrowski 1983; Socci and Templer 2011). While storage of nutrients within evergreenss has been examined, we have yet to link spatial and temporal patterns of N availability in soils to N uptake in mature trees in a field setting.

While soil nutrient dynamics influence plant nutrient uptake, storage, and use, plant functions also influence soil processes. This is especially noticeable in invaded ecosystems, where non-native species trigger positive feedbacks in nutrient cycling (Liao et al. 2008). For example, often times during plant invasion, total plant biomass increases,

positively influencing litter biomass, litter decomposition rates, and C and N stocks in soil organic matter. Then, net N transformation rates increase, leading to higher concentrations of ammonium and nitrate in the soil. The increase in soil N availability enhances net primary productivity (NPP), initiating further increases in biomass and driving the positive feedback between plant invasion and changes in soil nutrient dynamics (Ehrenfeld 2010). While general patterns of ecosystem change under plant invasion are established, some specific ecologically and culturally significant systems experiencing invasion have not yet been examined (Percy et al. 2016). Invaders under varying climate regimes may inflict unexpected changes in ecosystem dynamics, and establishing how specific ecosystems respond to land use change is important to inform management practices in those systems.

By combining techniques from biogeochemistry and plant physiology, I have explored basic physical and biological controls on nutrient dynamics. In chapter two, I explore how soil N availability varies in conjunction with N storage and allocation in *Pseudotsuga menziesii* growing in a montane forest of western Montana. In chapter three, I address how climate variability and plant invasion influence soil N and P dynamics in tropical montane forests of the Galapagos Archipelago. Because anthropogenic climate change, land use change, and invasion are three of the major global change processes that threaten temperate and tropical montane forests, understanding these basic process will become increasingly important as plant invasions intensify and as climate continues to change.

CHAPTER TWO

LINKING NITROGEN ALLOCATION IN DOUGLAS-FIR TO SOIL NITROGEN AVAILABILITY IN A WESTERN MONTANE CONIFER FOREST

Contribution of Authors and Co-Authors

Manuscript in Chapter 2

Author: Claire Qubain

Contributions: CAQ collected samples, performed chemical and statistical analyses, and wrote the first draft of the manuscript.

Co-Author: Yuriko Yano

Contributions: YY secured funding, developed the sampling protocol, collected samples, and provided feedback on the manuscript.

Co-Author: Jia Hu

Contributions: JH secured funding, developed the sampling protocol, helped with sample collection, and provided feedback on the manuscript.

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LINKING NITROGEN ALLOCATION IN DOUGLAS-FIR TO SOIL NITROGEN AVAILABILITY IN A WESTERN MONTANE CONIFER FOREST

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Abstract

It is widely accepted that temperate ecosystems are nitrogen (N) limited; thus, the magnitude of N available in the soil influences plant N uptake and assimilation. The main objective of this study was to explore whole tree N uptake dynamics across a season and to link N uptake with soil available N. We used a whole tree N mass balance approach to infer seasonal changes of total N between multiple needle and stem cohorts and bole tissue (referred to as growth fractions). Foliar nitrogen isotopes ($\delta^{15}N$) and net N transformation rates in the soil served as independent measurements to predict if trees were drawing from storage versus uptake to support their new needle growth. We detected that Douglas-fir (*Pseudotsuga menziesii* var. glauca (Mayr) Franco) trees

accumulated 51.09 ± 1.06 g N/ tree in 2016 and 89.40 ± 1.07 g N/ tree in 2017 in their new needle growth. However, no significant drawdown of N from previous years' growth fractions coincided with the accumulation of N, suggesting that the N in new needles was not reallocated from storage within the growth fractions tested. The depletion of foliar $\delta^{15}N$ before bud break suggested that mycorrhizae mediated uptake of N from the soil. However, Douglas-fir did not synchronize N uptake with periods of high soil N availability. Overall, our results demonstrated that mature Douglas-fir are drawing the bulk of their N from the soil, which indicates efficient use of available N in the ecosystem.

Introduction

It is widely accepted that temperate ecosystems are nitrogen (N) limited; thus, the magnitude of N available in the soil influences plant N uptake and assimilation (Chapin et al. 1988). N availability in soils, in turn, is controlled by numerous abiotic factors. In higher elevation western U.S. forests, snow is a dominant abiotic factor that drives N cycling. Throughout the winter, snow insulates the soil and allows soil temperatures to remain above freezing so that N ammonification and mineralization can occur (Maurer and Bowling 2014). As a result, N accumulates in the soils over winter because plants are not taking it up, and is subsequently released in a large pulse during snowmelt (Brooks et al. 1998). Given that N is an important nutrient for photosynthesis and growth, plants should take up this large pool of N as soon as it is readily available. However, conifers may not be using this readily available pool to support new growth (Nasholm and Ericsson 1990; Proe and Millard 1994). In one study, *Picea sitchensis* relied on N taken

up the previous autumn to support new growth before bud break, suggesting that an asynchrony could develop between when N is available versus when trees actually take up N to support new growth (Proe and Millard 1994). However, given that many of these studies were conducted in controlled greenhouses or plantations, it remains unclear how prevalent this asynchrony is under field settings where snow is the dominant form of precipitation.

While available N in the soil is relatively easy to measure across time, as demonstrated across a range of ecosystems, including tropical and temperate forests, grasslands, and arctic and alpine tundra systems (Vitousek and Matson 1988; Davidson et al. 1992; Turner et al. 1997; Jaeger et al. 1999; Schimel et al. 2004; Campbell et al. 2014), N uptake by plants remains more difficult to measure. The in-situ depletion method, where intact roots are immersed in a solution of known N concentration, can offer a direct measure of the magnitude of N uptake across a season, but the method can be time intensive and difficult to implement at a larger spatial scale. Alternatively, a mass balance approach helps to track changes in foliar nutrient concentrations across an entire year to assess if N accumulating in new tissues is translocated from parts of the plant or directly taken up by the roots. By accounting for changes in biomass in the branches of *Picea mariana* across a season, Chapin and Kedrowski (1983) found that by bud break, the current season's growth had already accumulated 70% of its maximum N concentration. Applying the mass balance approach at the whole tree level might allow us to determine if trees use newly acquired N or N from storage to support new growth.

While N uptake is difficult to measure directly, plant N uptake is mainly controlled by water availability and temperature. (Chapin and Kedrowski 1983; Gessler et al. 2002; Socci and Templer 2011). Nutrients move through the soil to the root interface mainly via diffusion, and when soils dry down, nutrient transfer to roots slows (Lambers et al. 1998; Matson et al. 2002). Similarly, low water availability constrains transpiration, indirectly inhibiting N uptake (Gessler et al. 2002). N uptake can also be inhibited by low soil temperatures and root damage in the spring (Millard and Grelet 2010; Sanders-DeMott et al. 2018). Thus, because N uptake is limited by temperature early in the growing season and by low soil moisture later in the season, stored N may be an important source supporting new growth. Furthermore, given that many plant storage and uptake studies have focused on tundra ecosystems and eastern U.S. deciduous forests, where midsummer moisture limitation rarely occurs, a different pattern of N storage and uptake may exist in ecosystems where mid-season moisture is limiting.

Plants may store N throughout different plant parts, including stems, branches, old leaves, new leaves, buds or roots. In evergreen plants, the main N storage pool is likely in old needles (Chapin 1980), in the form of protein, where it can represent up to 70% of total N (Millard 1988; Näsholm and Ericsson 1990). Rubisco, which makes up 50% of the total protein in C3 plants, is the main component of protein N storage (Chapin et al. 2011; Tegeder and Masclaux-Daubresse 2018). Along with protein storage, amino acids, such as arginine, asparagine, and glutamine, as well as ribosomal proteins, play a role in transient N storage across the season (Nasholm et al. 1994; Masclaux-Daubresse et al. 2017). However, it is still uncertain if these pools are always reallocated to support new

growth, particularly in evergreenss growing in snow-dominated ecosystems with dry midsummer conditions. The mass balance approach has been applied to track net changes in foliar N in order to detect storage mobilization within plants (Chapin 1980). If trees primarily use stored N to support new needle growth, then total N content in the past season needles, the branches, or the bole are expected to decline as new growth accumulates N throughout the season (Fig. 1.1a). However, if trees are primarily using newly acquired N from the soil, then N in the past season needles, the branches, or the bole are not expected to decline as new growth accumulates N throughout the season (Fig. 1.1b).

Given that N uptake, storage, and remobilization are important processes that regulate tree growth, refining our knowledge of these patterns is essential for our basic understanding of how mature trees use N. Thus, the main objective of this study was to explore whole tree N uptake dynamics across a season and to link N uptake with soil available N. More specifically, we asked the following questions: 1) How much N is remobilized from storage in evergreen trees versus how much N is newly acquired from the soil in order to support new growth? 2) Do these patterns of N remobilization versus uptake differ across an elevation gradient? 3) If trees do rely heavily on newly acquired N, do seasonal patterns of N uptake synchronize with seasonal patterns of soil available N? In order to address these questions, we applied an N mass balance approach in young (40 – 80 yrs.) Douglas-fir trees growing in western Montana to estimate changes in N of different growth fractions within an entire tree (Hansen et al. 1991). While this approach has been successfully demonstrated in shrubs (Chapin III et al. 1980; Gray and

Schlesinger 1983) and in trees grown on plantations (Nambiar and Fife 1991), this is one of the first studies to use this approach on whole trees in the field.

Methods

Site Description

The study was conducted during the 2016 and 2017 growing seasons in a mixed conifer forest of the North Fork of Elk Creek (NFEC) watershed at Lubrecht Experimental Forest in western Montana, USA. We sampled both trees and soils on southeast facing hillslopes at both a high (c. 1700 m) and a low (c. 1400 m) elevation site. The mean temperatures from 1981 to 2010 were 4.2 °C and 3.0 °C at the low and high elevation sites, respectively. Since 1970, snowfall represents 24% and 41% of annual precipitation at low and high elevations, respectively (NRCS SNOTEL, stations 604 and 657). The soil types ranged from extremely gravelly sandy loam and gravelly sandy loam to gravelly ashy loam, depending on sampling site and soil depth (NRCS, 2017). To record air temperature, relative humidity, and soil volumetric water content (VWC) at both sites, we used VP3 and 5TE sensors connected to EM50 data loggers recording at 30-minute intervals (METER group, Pullman, WA). To record precipitation, we used a tipping bucket rain gauge (METER, Pullman, WA). The data logger at the high elevation site malfunctioned between mid August and the end of September of 2017, so only low elevation data is reported for that period.

Growth fraction collection and analysis

In order to examine seasonal changes in percent N (%N), carbon and N ratio

(C:N), and $\delta^{15}N$ among different plant parts, we collected different growth fractions, including needles, buds, stems, litter, and bole tissue from 10 Douglas-fir throughout the 2016 and 2017 growing seasons. Five trees were located at the high elevation site and five were located at the low elevation site. In 2016, we sampled 13 times, starting in mid-April and ending in the beginning of November. In 2017, we sampled 12 times, starting at the end of February and ending in mid-September. During the February sampling period in 2017, only trees at the low elevation site were sampled because snow had not melted at the high elevation site, and trees there were considered dormant. Litter samples were collected using litter traps beginning in September 2016. During each sampling period, we collected one approximately 3.4 cm long bole sample using a tree borer. We also collected sun needles from three small branches in the middle canopy using a pole pruner (Fig. 1.2-1). Directly after clipping, we divided needles and stems by cohort (Fig. 1.2-2). In 2016, the samples were divided between buds or current year (CY) needles, (depending on the timing of bud break relative to sampling period), previous year (CY-1) needles, CY-1 stems, and CY stems if present. In 2017, we divided samples into buds or CY needles, CY-1 needles, two-year-old (CY-2) needles, CY-1 stems, CY-2 stems, and CY stems if present. New stem growth did not suberize until August in both years, so new stem growth was considered needle material until the August sampling date for both years. We determined the beginning and end of each needle and stem cohort by the annual bud-scale scars on the stems. If both buds and needles were present during a collection period, both growth fractions were collected for that period. Bud break occurred during the week of May 31 in 2016 and June 15 in 2017 at the low elevation

site, and during the week of June 15th in 2016 and June 25 in 2017 at the high elevation site.

After collection, all samples were transported back to the lab and dried at 60°C for 48 hours. All growth fractions except for the bole tissue were homogenized and hand ground using liquid nitrogen. Bole samples were ground in a cyclone sample mill (UDY corporation, Colorado, USA) and then pulverized using a tissue lyser (Quiagen TissueLyser II, Hilden, Germany) for 12 minutes. After all samples were ground and weighed, the needles and buds were analyzed for total N, total C, $\delta^{15}N$, and $\delta^{13}C$ content using continuous flow dual isotope analysis with a CHNOS Elemental Analyzer interfaced to an IsoPrime100 mass spectrometer at the University of California Berkeley (Fig.1.2-3). Because woody tissues were typically too low in N for accurate isotope analysis and because we did not think these fractions would be as dynamic across the growing seasons, stem, bole, and needle litter samples were only analyzed for total N and total C using a CHNOS Elemental Analyzer at Montana State University.

Mass balance: whole tree harvest and calculations

While most studies focus on using parameters such as percent N or C:N to infer seasonal patterns of N allocation, we used a whole tree N mass balance approach by multiplying percent N by the total estimated mass of the different growth fractions. In order to estimate total biomass of each growth fraction, at the end of July 2016, we harvested six trees, ranging from 7.5-15.8 cm diameter at breast height (DBH) (Fig 1.2-4). We divided subsets of six branches from each tree (three each from the upper and lower canopy) into separate growth fractions (bole, branches, CY needles, CY-1 needles,

CY stems, CY-1 stems, buds, all other needles, and all other stems) and reserved a subset of the bole. We weighed the entire cut tree in the field and then brought samples back to the lab to dry at 60 °C. The needles and stems were dried for one week and the boles were dried for two months.

Because we could not sample the entire tree, we estimated the mass of each growth fraction in a whole tree from a subset of branches, three upper canopy branches and three lower canopy branches) (Fig 1.2-4). From these six branches, we estimated the total dry mass of each growth fraction for an entire tree. We then developed an allometric relationship between DBH and each growth fraction's dry mass (Fig 1.2-5). We applied this allometric relationship to the 10 trees that we continuously sampled from (hereafter referred to as N-collection trees) to estimate the dry mass of CY needles, CY-1 needles, CY-1 stems, and bole tissue (Fig. 1.2-6). Because we only collected bole samples from the outer 3.4 cm, we divided the total predicted bole dry mass by the tissue sampled relative to the entire DBH of the tree. In order to calculate whole tree N content, we multiplied the estimated biomass of each growth fraction by the N concentration of each growth fraction from each sampling period. Dry mass estimates of CY-1 stems were applied to CY-1 stems in 2016 as well as CY-1 and CY-2 stems in 2017. Dry mass estimates of CY needles were applied to CY needles in 2016 as well as CY and CY-1 needles in 2017. Dry mass estimates of CY-1 needles were applied to CY-1 needles in 2016 and CY-2 needles in 2017.

We harvested trees in late July to ensure that new needles were fully elongated (maximum biomass); however, we needed to estimate the change in biomass as new

needles flushed. We used published data to estimate the percent of maximum elongation in new growth as a function of the number of days after bud break (Emmingham 1977), making the assumption that the trees in our environment followed similar patterns of bud break phenology as those in a central Cascades environment. To estimate bud dry mass, in March 2017, we collected one branch from the middle canopy of three separate trees at the same site where trees were harvested in July 2016. We divided the branches between CY buds, CY-1 needles, CY-1 stems, CY-2 needles, and CY-2 stems to record the wet mass of each fraction. After drying, we estimated the mean ratio of bud dry mass to CY-2[1] needle dry mass as 0.0757. We then calculated bud biomass estimates for the N-collection trees by multiplying the CY-1 biomass predicted from the allometric relationship times the bud dry mass ratio calculated.

Lastly, to scale litter N to the whole tree level, we estimated how many litter traps, which were approximately 0.13 m^2, could fit under each tree canopy and multiplied that area times the total dry biomass of each litter sample after each collection. The crown area of each tree was estimated using relationships developed between crown diameter and DBH following Curtis and Reukema (1970).

Soil N Sampling

To measure N availability, we used an ion exchange resin (IER) probe method, as well as the buried bag approach (Eno 1960). At both high and low elevation sites, every month, from April until August 2016, eight soil IER probes (4 cation and 4 anion probes)

[1] CY-2 needles sampled in 2017 and CY-1 needles sampled in 2016 are the same needle cohort that flushed in 2015.

were inserted at six locations on the same hillslopes where sampled trees were growing. Additionally, monthly from April through October 2017, we inserted four IER probes (2 cation and 2 anion probes) and one buried bag of soil under the canopy of each N-collection tree. After removal, the probes were kept cool and cleaned with distilled water within 48 hours, refrigerated until the end of the season, and then analyzed for NH_4-N and NO_3-N using flow injection analysis (Western Ag Inc.).

To carry out the buried bag approach, we collected pairs of soil samples to a 15 cm depth from under each N-collection tree from May until October 2017. One of the samples was buried in a plastic bag under the canopy for a one-month incubation period, and the other was cooled and transported back to the lab for processing. After the month long incubation period was over, we removed the buried samples. To process both sets of samples, we extracted the samples using 1M KCl within 48 hours of sampling. Extracts were gravity filtered through p8 coarse paper filters then syringe filtered through 0.7 um glass filter tips. After filtration, the samples were analyzed for ammonium (NH_4^+) and nitrate (NO_3^-) using flow injection analysis (Lachat Quik-Chem Series 2400, Colorado).

Statistical Analysis

For the biomass scaling, we fit linear models to each separate growth fraction in R (R Core Team 2017). We tested how dry mass in CY needles, CY-1 needles, CY-1 stems, and boles varied as a function of DBH. In these models, and all following models, normal-QQ plots and residual plots were used to visually assess how each model met the assumptions of normality and constant variance, and both CY needle dry mass and CY-1 needle dry mass were log transformed to meet those assumptions. One outlier was

removed from the CY-1 stem model because it had a Cook's distance value greater than one.

For the %N, total N, C:N, and $\delta^{15}N$ response variables, we fit linear mixed effects models using the lme function in R (Pinheiro et al. 2014; R Core Team 2017). To select each model, we started with full models of three way interactions between fixed effects of a second order polynomial of days after flush (where negative values were before bud break and positive values were after bud break each growing season), growth fraction, and elevation, and a random effect of individual tree. A parameter or interaction was not included in the model if it had a p-value > 0.05. After using this stepwise model selection process, we presented models testing how each response variable (%N, C:N, total N, and $\delta^{15}N$ changed as a function of an interaction between a 2nd order polynomial of days after flush and growth fraction, after controlling for the random effect of individual trees. All response variables except $\delta^{15}N$ were log transformed to meet the assumption of constant variance. In 19 bole samples, %N was too low to measure using the elemental analyzer, so those samples were treated as not available (NAs) in the model. To report contrasts in the response for each growth fractions across time, we used the predict.lme function (Pinheiro et al. 2014).

We used linear mixed effects models to separately examine NH_4^+ and NO_3^- net transformation rates collected using both the buried bag and IER probe methods. In each model, we tested how the log transformation of both NH_4^+ and NO_3^- varied as a function of time and elevation, after accounting for the random effect of sampling location. The N transformation rates using the IER probe method were modeled separately for 2016 and

2017 because the probes were collected from two different locations, and the net N transformation rates using the buried bag approach were only collected and analyzed for the 2017 season.

Results

Growth fraction %N and C:N

The timing of snowmelt between the high and the low elevation sites can differ by almost one month (Yano et al., Under Review), leading to differences in the timing of bud break. Therefore, in order to compare the %N and C:N ratios of the different growth fractions, we normalized time on the x-axis to reflect before and after bud-break. We first examined differences in %N between the two different elevations using the following linear mixed effects model:

$$\log(\%N \text{ of growth fractions sampled}) = ß_0 + ß_1 time + ß_2 time^2 + ß_3 I_{g=CYn} + ß_4 I_{g=CYbd} + ß_5 I_{g=CY-1n} + ß_6 I_{g=CY-1s} + ß_7 I_{g=CY-2n} + ß_8 I_{growth=CY-2s} + ß_9 I_{g=l} + ß_{10} I_{g=b} + ß_{11} time^2 * I_{g=CYn} + ß_{12} time^2 * I_{g=CYbd} + ß_{13} time^2 * I_{g=CY-1n} + ß_{14} time^2 * I_{g=CY-1s} + ß_{15} time^2 * I_{g=CY-2n} + ß_{16} time^2 * I_{growth = CY-2s} + ß_{17} time^2 * I_{g=l} + ß_{18} time^2 * I_{g=b} + ß_{19} I_{elev = low} + ß_{20} I_{elev = high} + tree_i + \varepsilon_{ij},$$
$$Tree_i \sim N(0, \sigma^2), \varepsilon_{ij} \sim N(0, , \sigma^2_{\varepsilon})$$

where g = growth fraction, CY = current year, n = needle, bd =bud, s = stem, l = litter, b = bole, and elev = elevation, ε_{ij} = residual variability of the j^{th} occasion for the i^{th} subject, and N (0, σ^2) denotes that errors are normally distributed with a mean of 0 and a variance of σ^2.

Each year was modeled separately to better meet the assumptions of normality and constant variance. We found that median %N in all the growth fractions was 1.13% higher at the high elevation than at the low elevation site in both 2016 (χ^2 (1, 8 D.F.) = 35.48, p-value < 0.001) and 2017 (χ^2 (1, 8 D.F.) = 23.17, p < 0.0001). Furthermore, median %N in each growth fraction was dependent on the number of days after bud break (χ^2 (12, 518 D.F.) = 27.38, p-value < 0.0001 in 2016 and χ^2 (14, 737 D.F.) = 19.37, p-

value < 0.0001 in 2017). Among the different growth fractions, %N was highest in buds and CY needles during the bud break period, followed by CY-1 needles and CY-2 needles, and CY-1 stems (Fig. 1.4). Lowest %N was found in bole tissue, litter, and CY-2 stems (Fig. 1.4).

As trees increase biomass in the spring while also acquiring N to support new growth, stoichiometric variability (e.g. C:N) among different growth fractions may provide insight into the controls of biomass on N concentrations. We used the following linear mixed effects model:

$$\log(C:N) = ß_0 + ß_1 time + ß_2 time^2 + ß_3 I_{g=CYn} + ß_4 I_{g=CYbd} + ß_5 I_{g=CY-1n} + ß_6 I_{g=CY-1s} + ß_7 I_{g=CY-2n} + ß_8 I_{growth=CY-2s} + ß_9 I_{g=l} + ß_{10} I_{g=b} + ß_{11} time^2 * I_{g=CYn} + ß_{12} time^2 * I_{g=CYbd} + ß_{13} time^2 * I_{g=CY-1n} + ß_{14} time^2 * I_{g=CY-1s} + ß_{15} time^2 * I_{g=CY-2n} + ß_{16} time^2 * I_{growth = CY-2s} + ß_{17} time^2 * I_{g=l} + ß_{18} time^2 * I_{g=b} + ß_{19} I_{elev = low} + ß_{20} I_{elev = high} + tree_i + \varepsilon_{ij}, Tree_i \sim N(0, \sigma^2), \varepsilon_{ij} \sim N(0, , \sigma^2_\varepsilon)$$

Each year was modeled separately to better meet the assumptions of normality and constant variance. We first examined differences in C:N between the high and the low elevation and found that the absolute C:N ratio was 0.89 greater at the high elevation than at the low elevation in 2016 (95% C.I. of 0.85 to 0.93) and 0.91 greater (95% CI of 0.87 to 0.95) in 2017 after accounting for growth fraction and time. We also examined C:N among the different growth fractions and found that similar to %N, the C:N ratio in each growth fraction was dependent on the number of days after bud break (χ^2 (12, 534 D.F.) = 22.80, p-value < 0.0001 in 2016 and χ^2 (14, 746 D.F.) = 22.02, p-value < 0.0001 in 2017). In both years, the bole had the highest C:N ratio, followed by litter, CY-2 stems, CY-1 stems, CY-2 needles, and CY-1 needles (Fig. 1.5). Across the year, bud C:N declined prior to bud break, reaching the lowest point just prior to bud break, but then increased

following bud break; this suggests that the addition of new biomass following bud break diluted the N signal. The C:N ratio in bole tissue also declined before and after bud break, and C:N in CY-1, and to a lesser degree in CY-2 stems, increased (Table 1.1).

Mass balance: whole tree harvest and calculations

From our whole tree harvests, we developed allometric relationships between DBH and mass of the different growth fractions (Fig. 1.5). Strong evidence suggested that the dry mass of CY needles, CY-1 stems, and bole tissue varied as a function of DBH, but weak evidence suggested that the dry mass of CY-1 needles varied according to DBH (Table 1.1). For every 1 cm increase in DBH, dry biomass was predicted to increase by 1.40 Kg in CY needles (95% CIs of 1.09 – 1.79), 1.35 Kg in CY-1 needles (95% CIs of 90.09 to 1.98), 0.12 Kg in CY-1 stems (95% CIs of 0.02 to 0.22), and 4.0 Kg in bole tissue (95% CIs of 1.2 to 6.48 Kg).

Using a mass balance approach, we followed pools of N among different growth fractions within an entire tree throughout two growing seasons. We used the following linear mixed effects model:

$$\log(\text{Total N}) = ß_0 + ß_1\text{time} + ß_2\text{time}^2 + ß_3I_{g=CYn} + ß_4I_{g=CYbd} + ß_5I_{g=CY\text{-}1n} + ß_6I_{g=CY\text{-}1s} + ß_7I_{g=CY\text{-}2n} + ß_8I_{growth=CY\text{-}2s} + ß_9I_{g=l} + ß_{10}I_{g=b} + ß_{11}\text{time}^2 * I_{g=CYn} + ß_{12}\text{time}^2 * I_{g=CYbd} + ß_{13}\text{time}^2 * I_{g=CY\text{-}1n} + ß_{14}\text{time}^2 * I_{g=CY\text{-}1s} + ß_{15}\text{time}^2 * I_{g=CY\text{-}2n} + ß_{16}\text{time}^2 * I_{growth\,=\,CY\text{-}2s} + ß_{17}\text{time}^2 * I_{g=l} + ß_{18}\text{time}^2 * I_{g\,=b} + ß_{19}I_{elev\,=\,low} + ß_{20}I_{elev\,=\,high} + \text{tree}_i + \varepsilon_{ij},\ \text{Tree}_i \sim N(0, \sigma^2),\ \varepsilon_{ij} \sim N(0, \sigma^2_\varepsilon)$$

Each year was modeled separately to better meet the assumptions of normality and constant variance. Overall, after accounting for an interaction between days after bud break and growth fraction, and a random effect of individual tree, we did not find a difference in total N between elevations (p-value > 0.05) as we did for %N and C:N.

However, similar to the %N results, we found that differences in total N in each growth fraction were dependent on the day after bud break (χ^2 (12, 516 D.F.) = 187.97, p-value < 0.0001 in 2016 and χ^2 (14, 737 D.F.) = 73.89, p-value < 0.0001 in 2017). Before bud break in 2016, CY-1 needles maintained the largest N pool, but after bud break, the CY needles accumulated the most N out of all the growth fractions (Fig. 1.6). In 2017, most N was in CY-1 needles prior to bud break. However, unlike in 2016, total N in CY needles post bud break never surpassed that in CY-1 needles, even after CY needles in 2017 had accumulated 100% of their N. For the other growth fractions, total N did not significantly change before and after bud break, although there were differences in total N between the different growth fractions. We found highest total N in CY needles, followed by CY-1 and CY-2 needles, bole tissue, CY-1 stems, CY-2, and buds. We calculated that post bud break, CY needles accumulated a median of 51.09 ± 1.06 g N/ tree in 2016 and 89.40 ± 1.07x g N/ tree in 2017.

Isotopic approach to estimate uptake versus reallocation

We also used an isotope approach as an independent method to support our estimations of uptake versus reallocation of N by analyzing the $\delta^{15}N$ of buds and needles. We used the following linear mixed effects model:

$$\delta^{15}N = ß_0 + ß_1 time + ß_2 time^2 + ß_3 I_{g=CYn} + ß_4 I_{g=CYbd} + ß_5 I_{g=CY-1n} + ß_6 I_{g=CY-2n} + ß_7 time^2 * I_{g=CYn} + ß_8 time^2 * I_{g=CYbd} + ß_9 time^2 * I_{g=CY-1n} + ß10 time^2 * I_{g=CY-2n} + ß_{11} I_{elev = low} + ß_{12} I_{elev = high} + tree_i + \varepsilon_{ij}, Tree_i \sim N(0, \sigma^2), \varepsilon_{ij} \sim N(0, \sigma^2_\varepsilon)$$

We did not detect a difference in mean $\delta^{15}N$ between elevations (χ^2 (1, 8 D.F.) = 4.36, p-value = 0.07). However, significant differences in $\delta^{15}N$ in each growth fraction were

dependent on the day after bud break (χ^2 (6, 601 D.F.) = 14.16, p-value < 0.0001). For both CY and CY-1 needles, $\delta^{15}N$ began to decline prior to bud break, but then increased sharply around the period of bud break and new needle flush. We did not find differences in mean $\delta^{15}N$ between needle cohorts, except at the low elevation in 2017, where buds were more depleted than CY-1 and CY-2 needles (Fig. 1.7).

Soil N Mineralization & Nitrification

Using a linear mixed model, we tested how transformations of NO_3^- and NH_4^+ responded as a function of burial period and site, after accounting for a random effect of the sampling location. From the IER probes, we found a difference in NO_3-N across the seasons in 2016 (χ^2 (1, 11 D.F.) = 11.2, p-value = 0.006) and in 2017 (χ^2 (1, 49 D.F.) = 6.70, p = 0.01); we found a difference between elevations in 2017 (χ^2 (1,8 D.F.) = 8.30, p-value = 0.02 in 2017), but not in 2016 (p-value = 0.8 in 2016). After every additional burial period, the rate of NO_3-N nitrification increased by 1.02 mg/m^2/month in 2016 and 1.0 mg/m^2/month in 2017 (95% C.I. of 1.01 to 1.04 mg/m^2/month in 2016 and 1.01 to 1.15 mg/m^2/month in 2017). In 2017, NO_3-N was 2.01 mg/m^2/month higher at the high elevation site compared to the low elevation site (95% CI of 1.15 to 3.66 mg/m^2/month).

From the IER probes, we detected a difference in NH_4-N across time in 2017 (χ^2 (1,49) = 10.42, p-value = 0.002 in 2017), but not in 2016 (χ^2 (2, 10) = 0.8, p-value = 0.4 in 2016), and no differences between elevations in 2016 or 2017 (χ^2 (1,4) = 0.81, p-value = 0.4 in 2016, χ^2 (1,8) = 4.09, p-value = 0.07 in 2017). For every additional burial period,

the median NH_4-N increased by 2.05 mg/m^2/month in 2017 (95% CI 1.15 to 3.65 mg/m^2/month), and it was 2.34 mg/m^2/month greater at the high elevation compared to the low elevation site in 2017 only (95% CI of 0.88 to 6.16 mg/m^2/month).

From the buried bag method implemented in 2017, net NO_3-N and NH_4-N transformations were not different across time or between elevations (p-value = 0.2 and 0.6, respectively, for NO_3-N, and p-values = 0.95 and 0.73, respectively, for NH_4-N).

Discussion

N is a limiting nutrient for many temperate plants, and evergreens can potentially store large quantities, allowing them to be buffered from inter-annual variation in N availability (Warren and Adams 2004; Du et al. 2018). However, in our study, we found that Douglas-fir used N acquired from belowground during the present growing seasons, not from storage in older growth fractions, to support new needles. Tissue N concentrations and C:N ratios in separate growth fractions were lower in trees growing at a lower elevation, but scaling nutrient content to the whole tree level removed any elevation difference found in foliar N concentrations, which was just a function biomass in whole trees. Patterns of seasonal N uptake seemed to synchronize with periods of high N availability in the soil, as determined by observing foliar $\delta^{15}N$ and soil N availability across the growing seasons.

Leaf Level Measurements

Biomass accumulation, resulting from needle expansion during the growing season,

was the most important factor controlling changes in foliar %N and C:N ratios. Biomass accumulation is important because new needles become a carbon source for the rest of the tree for the remainder of the growing season. In buds, %N increased until bud break, when buds became CY needles and %N decreased, likely due to dilution by the addition of biomass across the seasons (Chapin and Kedrowski 1983; Fife and Nambiar 1984) (Fig. 1.4). C:N decreased in buds until bud break and then increased in CY needles until the end of the season, further demonstrating that the addition of C diluted the foliar N signal as needles flushed (Fig. 1.5).

Our findings are in line with reported N concentrations in studies of other evergreens. Percent N in needles from *Pinus radiata* seedlings decreased from 2.6% at bud break to 0.8% when they were three years old (Fife and Nambiar 1982). Though minimum %N in our study was around 1%, we still observed maximum values near those in the *Pinus radiata* study directly after bud break. Our findings of %N in all the remaining needle cohorts were about 0.5% greater than *Pseudotsuga menziesii* saplings raised in growth chambers (Hobbie et al. 2001). Compared with *Ledum palustre,* an evergreen shrub observed in taiga field settings, %N in our observations was 1.5% lower in CY needles directly after bud break, but that cohort maintained similar dilution by C across the growing season (Chapin et al. 1980). In the same study, %N in CY-1 needles and CY-1 stems were 0.5% and 1.0% higher, respectively, across the season than presented here. In general, foliar nutrient concentrations fell within the range of 10 mg g^{-1} to 25 mg g^{-1} found for evergreens summarized from 92 studies (Aerts and Chapin 1999).

Whole Tree Nitrogen dynamics

The storage of resources, including nutrients, is an important plant function; however, some studies have found that evergreen species depend less on stored N and instead rely on N reabsorbed from litter or newly acquired N from the soil (Chapin 1980; Aerts and Chapin1999). Because total N did not decline in older needle and stem cohorts or in bole tissue, we determined that most of the N supporting new growth in Douglas-fir was not drawn from N stored in those growth fractions. Instead, our results suggest that the trees were using newly acquired N from the soil to support new growth.

Evergreens do not always draw down N storage from old leaves to support new growth (Chapin et al. 1990; Aerts and Chapin1999). In a tundra ecosystem, while 27 – 41% of the total N in new leaves of deciduous shrubs was drawn from storage, none of the total N in the new growth of an evergreen shrub, *Ledum palustre*, was drawn from storage in past season needles (Chapin et al. 1980), but no speculation as to where N was provided from was presented. In a similar study, even after old leaves were manually defoliated from evergreen species in tundra and Mediterranean environments, N accumulation in new leaves still continued (Jonasson 1989), further demonstrating that N to support new growth in evergreens was not derived from N stored in old leaves.

On the other hand, some studies have shown that evergreenss allocate N from storage to support the current season's growth, indicating an uncoupling of foliar N from soil nutrient variability across seasons. For example, *Ceanothus megacarpus*, an evergreen shrub, used stored N to avoid nutrient limitation during soil dry down or when soil nutrients were not readily available (Gray 1983; Gray and Schlesinger 1983). *Ceanothus* even stored more N than deciduous species (Chapin III and Shaver 1989), which is

uncommon, though this could be due to the species' N-fixation strategy (Gray 1983). From a greenhouse study using an isotope tracer method, *Picea sitchensis* seedlings used N from the previous season to support current season needles (Proe and Millard 1994). Lastly, using a mass balance approach, Chapin and Kedrowski (1983) observed that old needle N in *Picea mariana* decreased throughout the summer and increased again by winter, indicating that those trees were using and then replenishing stored N, 70% of which had already accumulated before bud break (Chapin and Kedrowski 1983). Differences in storage versus uptake patterns between this study and others are mostly likely due to differences in climate, growth habit, and life stages.

The mass balance approach has limitations (Nambiar and Fife 1991; Proe and Millard 1994). The method only allows for accounting net nutrient transfer within the plant, not partitioning of N between each needle or stem cohort, potentially confounding the detection of a draw down in N storage (Proe and Millard 1994). Secondly, if stored instead of newly acquired N is supporting growth, the time when the stored N was taken up can not be detected. This study also has specific limitations. We assumed that roots and needles older than three years provided minimal N to support new growth because total N in *Pinus radiata* roots did not fluctuate seasonally (Nambiar 1987), and because nutrient pools in older needles are relatively inert (Aerts and Chapin III 1999). Some N, however, could have been reallocated from roots or needles and stems older than three years that we did not detect. Secondly, there were uncertainties surrounding our whole tree biomass estimates. Because we only estimated biomass at one point in time, we had to assume that the same amount of new biomass was added in 2017 as it was in 2016,

including bud biomass, and that new needle elongation occurred at the same rate as published values from tree populations from the central Cascade Mountains. Because Douglas-fir needles live for six to eight years (Balster and Marshall 2000), we also assumed no needle abscission occurred in CY-1 and CY-2 needles, likely leading to some overestimation of total N in those fractions. Despite the assumptions made, the mass balance approach still allowed us to estimate how much N mature trees growing under field conditions accumulate across two growing seasons.

Spruce Budworm Outbreak

The year before study began, a western spruce budworm (*Choristoneura occidentalis*) outbreak occurred across forests in the state of Montana, and a large percentage of the CY-1 needles in 2016 were defoliated (Qubain, C., *personal observation*). Spruce budworm can defoliate between 8% and 17% of a tree's gross volume in a given outbreak (Alfaro et al. 1985), and outbreaks are most severe in drought-stressed trees with relatively low foliar N concentrations (Cates et al. 1983; Redak and Cates 1984). During the season prior to our first year of sampling (2015), defoliation from spruce budworm was severe, and during our two sampling seasons (2016 and 2017), defoliation was present but minimal. The outbreak could have affected our conclusions regarding tree N origins across growing seasons. We predicted that if trees were drawing stored N to support new growth from the previous season's needles,

then we would detect little draw down in total N in CY-1 needles after severe defoliation (2016) but significant draw down in the same needle cohort after mild defoliation (2017). This pattern would suggest that the trees were not accessing N storage because that cohort was nearly gone after the severe outbreak, not because the trees do not use stored N in an undisturbed state, as we discovered. After not detecting a draw down of total N in CY-1 needles following the severe *or* the mild defoliation events, and knowing that artificial defoliation did not affect the use of stored N in *Pinus radiata* (Jonasson 1989), we concluded that budworm defoliation did not influence whether the trees studied here were using stored N or not. However, because total N in CY-1 needles during 2016 was so much lower than in 2017 (Fig. 1.7), we did determine that the outbreak affected the total size of the N pool in that needle cohort and could have affected the trees' ability to fix C.

Foliar $\delta^{15}N$ to predict storage versus uptake

Using an alternative and independent isotope method to assess N acquisition by Douglas-fir trees, we found further support that trees were using newly acquired N from the soil to support new growth. We observed that foliar $\delta^{15}N$ became more negative between the beginning of the season and bud break, and then became more positive between bud break and the end of the growing season (Fig. 1.8). Though naturally abundant ^{15}N is notoriously difficult to interpret (Hogberg 1997), the consistent temporal pattern in foliar $\delta^{15}N$ fractionation between needle cohorts, elevations, and seasons we detected indicates a clear pattern of N uptake. The temporal patterns in foliar $\delta^{15}N$

fractionation likely reflect mycorrhizal mediation of N from the soil, where mycorrhizae were readily transferring N to the trees before bud break and then slowed the transfer between bud break and the end of the season After uptake from the soil, mycorrhizae incorporate the lighter isotope into proteins and amino acids because it is kinetically easier to use a lighter molecule. The N transferred to the plant from the mycorrhizae is relatively depelted in ^{15}N, which is expressed in the foliar N (Hobbie and Högberg 2012). This finding is supported by a study examining mycorrhizal presence in a similar forest community in western Montana, where ectomycorrhizal root tips were most abundant in May and June (around the timing of the depletion event in this study) and least abundant in July and August (which correlates with the enrichment event after bud break here) (Harvey et al. 1978). Fractionation does not occur during plant uptake from bulk soil when N concentrations are low, as they were here, or when mycorrhizae are not present or active, so we would not have detected uptake without mycorrhizal mediation (Handley and Raven 1992; E. Dawson et al. 2002; Hobbie and Högberg 2012). While reallocation of stored N from old to new leaves in deciduous species did cause fractionation (E. Dawson et al. 2002; Kolb and Evans 2002), we did not detect a movement of stored N in this study, so we assumed that no fractionation occurred within the tree once the N was acquired from the soil.

Without examining mycorrhizal activity directly, other factors, such as variation in rooting depth or uptake of different forms of N could have contributed to variation in foliar $\delta^{15}N$ in this study (E. Dawson et al. 2002). However, these processes are unlikely to have occurred. Douglas-fir trees at this site use water from the upper meter of the soil

profile (Martin, J.T., Personal Communication), and because nutrient uptake is so closely linked to water use, the trees studied are likely drawing nutrients from the upper meter or higher of the soil. Secondly, because plants take up the form of N that is most readily available in the soil (Lambers et al. 1998), and NH_4^+ is an order of magnitude more concentrated than NO_3^- at this site (Yano et. al. Under Review), NH_4^+ is likely the main N form used across the season. So, variation in rooting depth and the form of N used from the soil likely had little influence on the temporal variation in the $\delta^{15}N$ patterns we observed across the seasons.

Soil N and climate conditions

Concentrations of N belowground must be high enough to supply the trees with their estimated annual N budget. The buried bag approach indicated that microbial immobilization of N was more dominant than mineralization in this ecosystem (Fig. 1.9). Further, the rate of NH_4^+ and NO_3^- adsorption to ion exchange resins increased across the growing season, indicating that mineralization rates were highest towards the end of the growing season and lowest during the beginning of the season (Binkley and Matson 1983; Krause and Ramalal 1987). Thus, soil N availability and Douglas-fir N accumulation did not synchronize. Nevertheless, they were still able to accumulate most of their N from belowground, even when N availability was low.

In conclusion, our findings suggest that evergreen trees growing in snow-dominated ecosystems rely upon N taken up from the soil during the present growing season to support new needle growth. While soil N was adequate to support the trees' accumulation of N in new growth, they did not synchronize soil N availability with N

uptake. By accessing N from the soils, trees preclude the loss of nutrients from the system they grow in, maintaining relatively closed cycling of N within the watershed.

Acknowledgements

Thank you to technicians E. Anderson, T. Simpson, C. Dart and C. Moss for their hard work collecting and processing samples. Thank you to J. Klassen for her guidance with chemical analysis.

Tables

Growth Fraction	Equation	F-stat	D.F.	P-Value	R2
CY Needles	$Log(Dry\ Mass) = ß_0 + ß_1 DBH$	14.76	1, 4	0.01	0.78
CY-1 Needles	$Log(Dry\ Mass) = ß_0 + ß_1 DBH$	5.06	1, 4	0.08	0.56
CY-1 Stems	$Dry\ Mass = ß_0 + ß_1 DBH$	15.5	1, 3	0.02	0.83
Bole	$Dry\ Mass = ß_0 + ß_1 DBH$	20.09	1, 4	0.01	0.83

Table 1.1 – Output from linear models used to predict the biomass of each growth fraction in the N-collection trees. CY stands for current year and DBH stands for diameter at breast height.

Figures

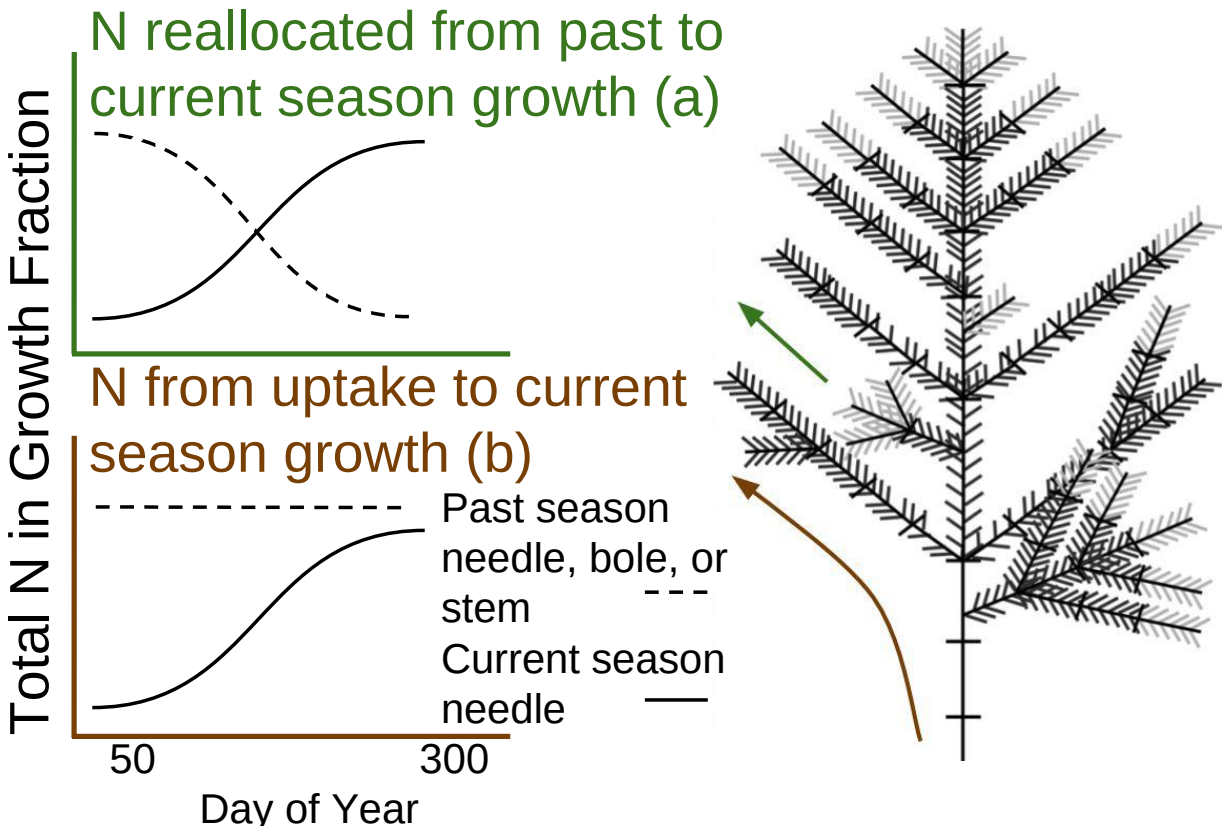

Figure 1.1 – Conceptual figure of potential expected outcomes, where a) represents a drawdown of stored N from the past season growth and b) represents that the tree bypasses storage and instead uses newly acquired N from the soil.

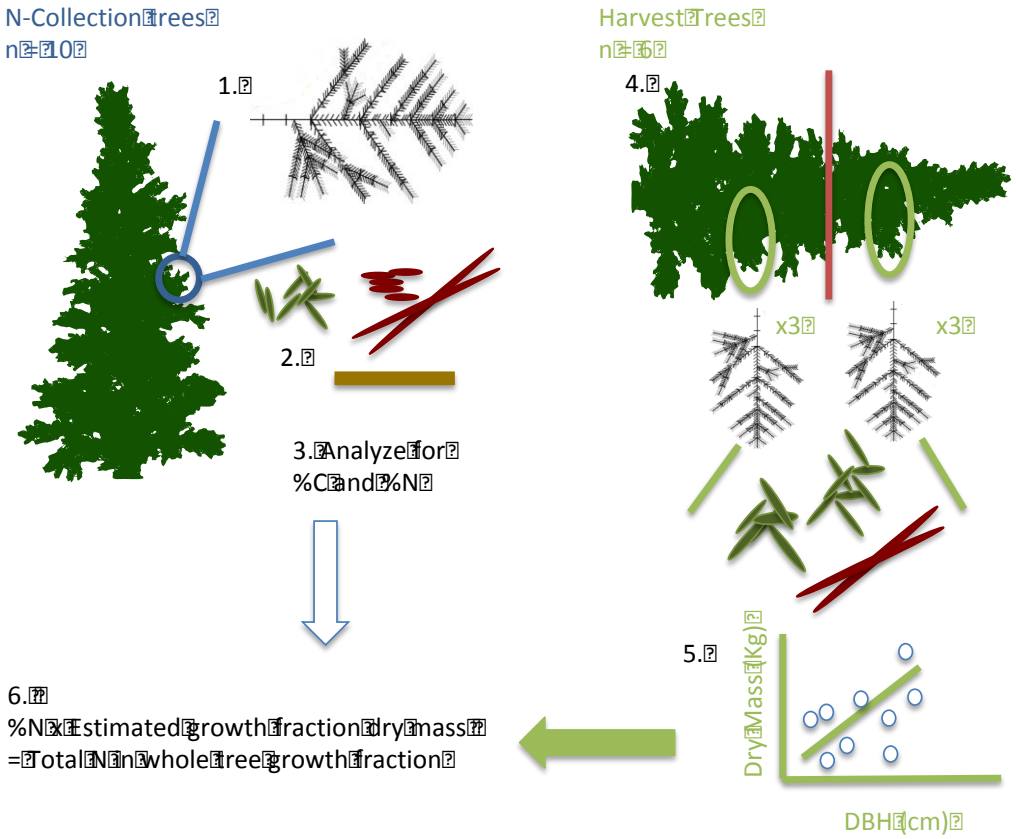

Figure 1.2 – Biomass harvesting methods used to calculate whole tree total N.

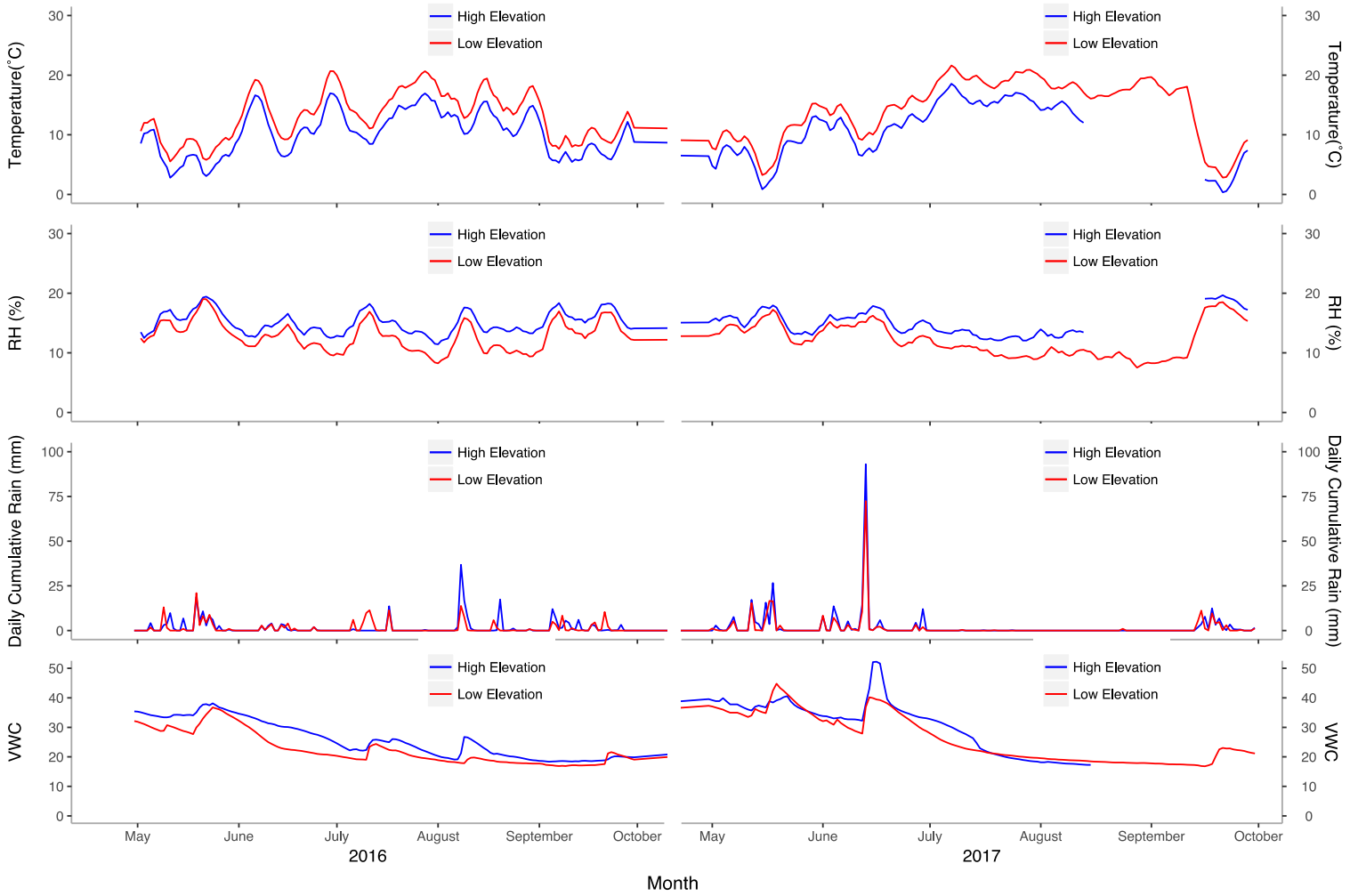

Figure 1.3 – 5-day moving averages of temperature (˚C) and relative humidity (%) are shown in the top two panels, and daily cumulative precipitation (mm) and the daily volumetric water content (VWC) averaged by 10 cm, 30 cm, and 50 cm soil depths are shown in the bottom two panel. The broken x-axis between years represents the overwinter period.

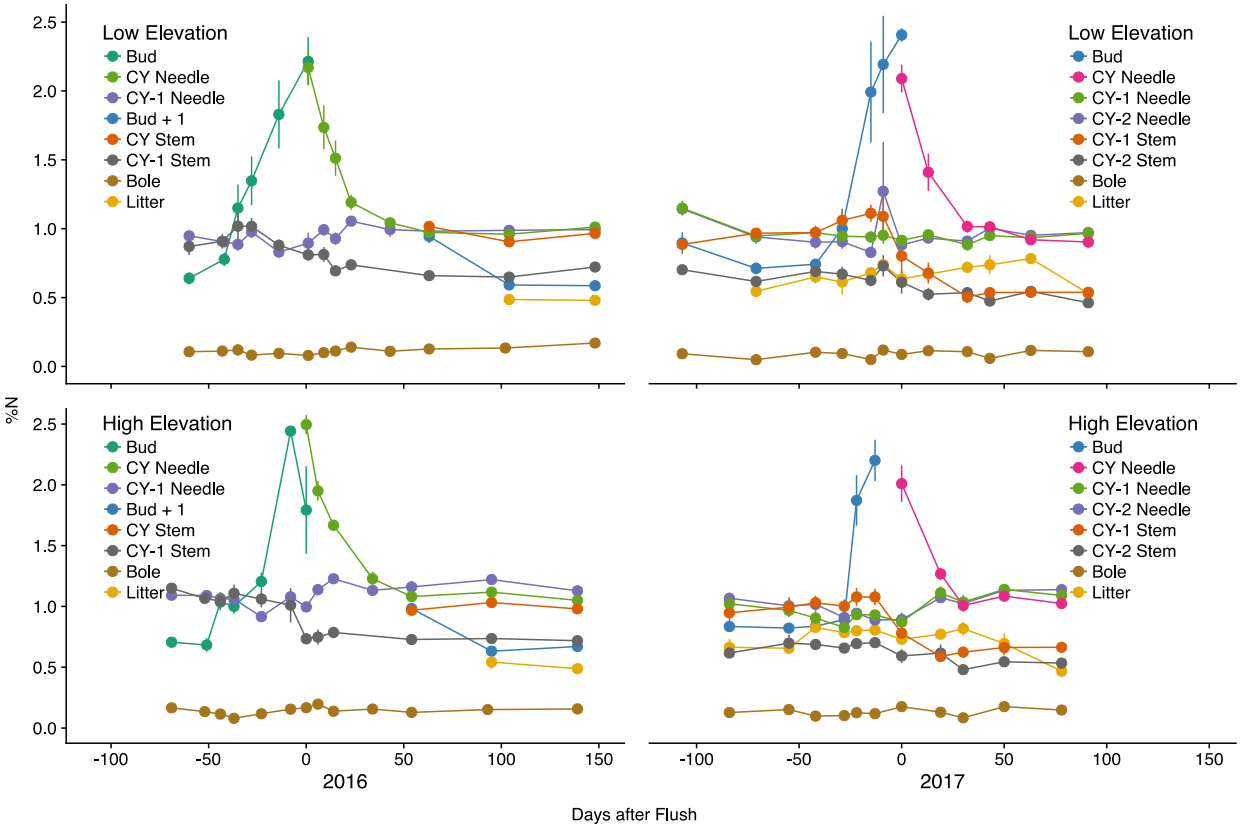

Figure 1.4 - Tissue %N is represented across time relative to bud break (n = 5) with standard errors. The top panel presents the changes of %N in separate growth fractions at the low elevation site, and the bottom panel presents the changes of %N at the high elevation site. Day 0 represents the day of bud break.

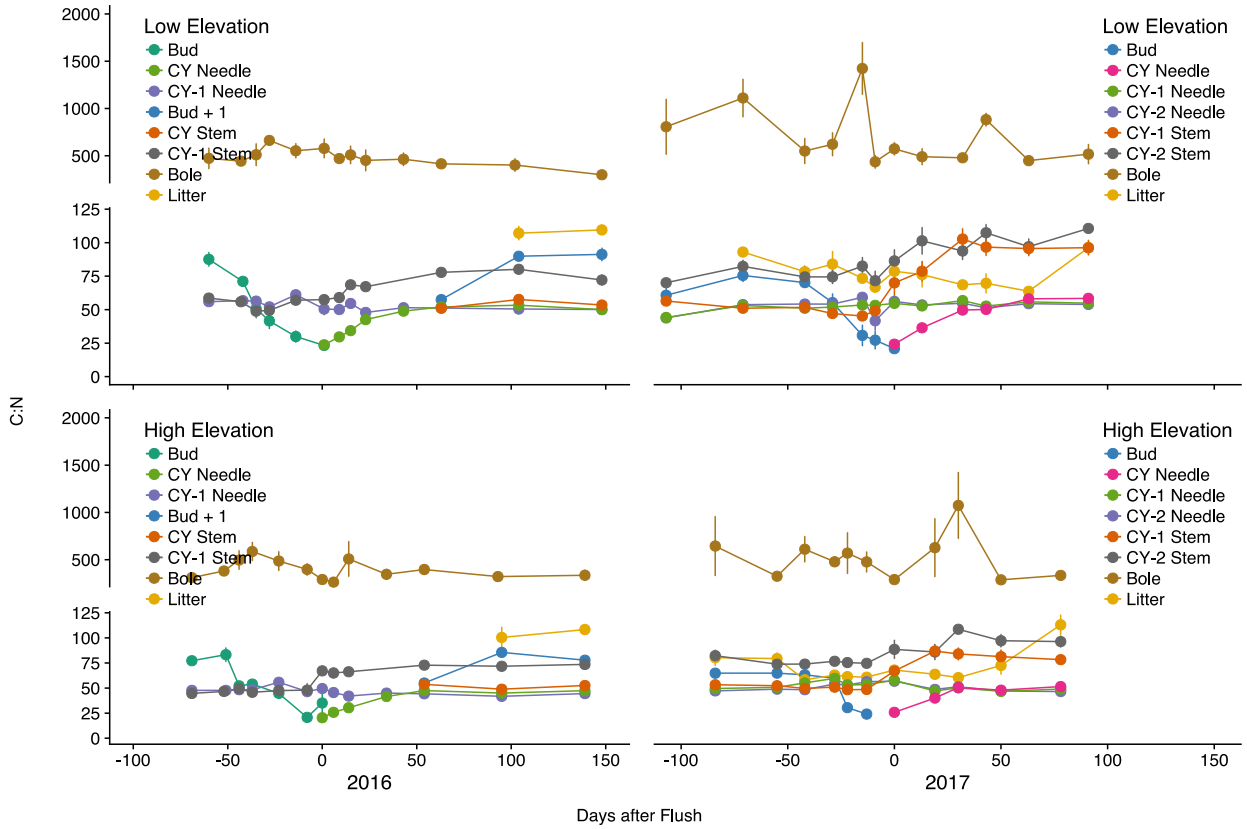

Figure 1.5 – C:N at the low elevation site (top panel) and at the high elevation site (bottom panel) show that dilution of N from an increase in biomass, C, occurs as new needles grow. Points are mean of 5 ratios, and error bars represent the standard errors. Note the split y-axis and different y-axis scales between the bole tissue and all other growth fractions.

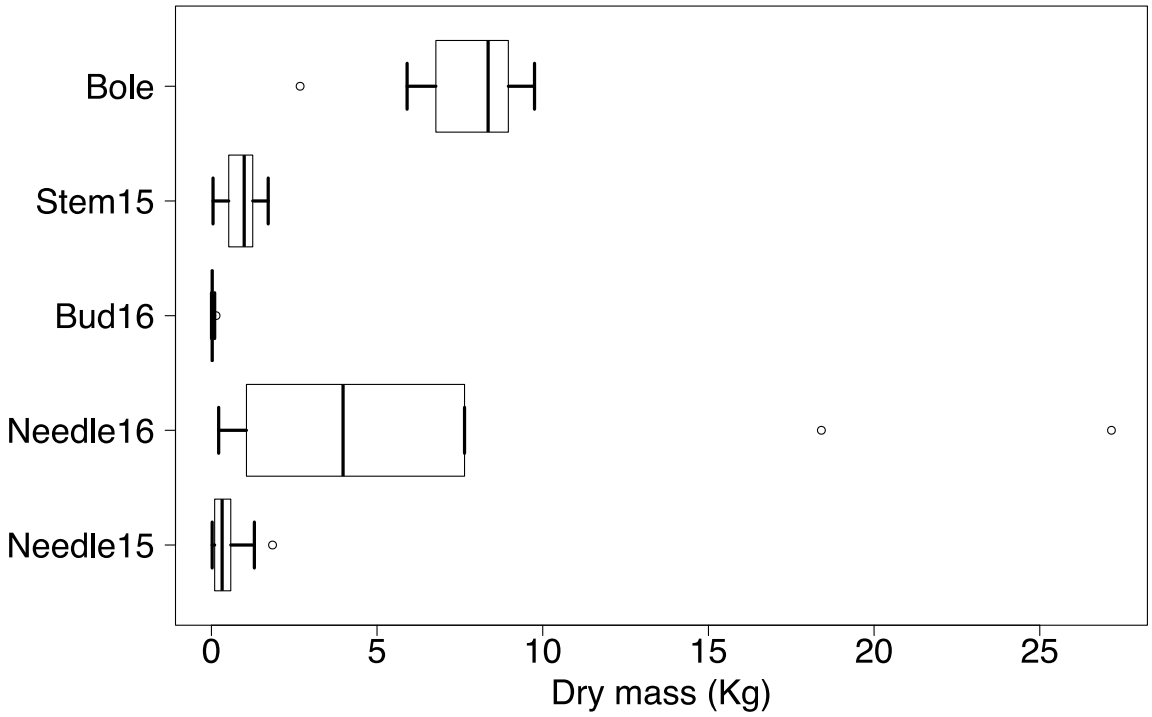

Figure 1.6 – Predicted biomass of each growth fraction in the trees that N was sampled. Values were extracted from the allometric relationships developed from growth fraction biomass as a function of diameter at breast height. Note: total biomass for the bole only represents the outer 3.4 cm).

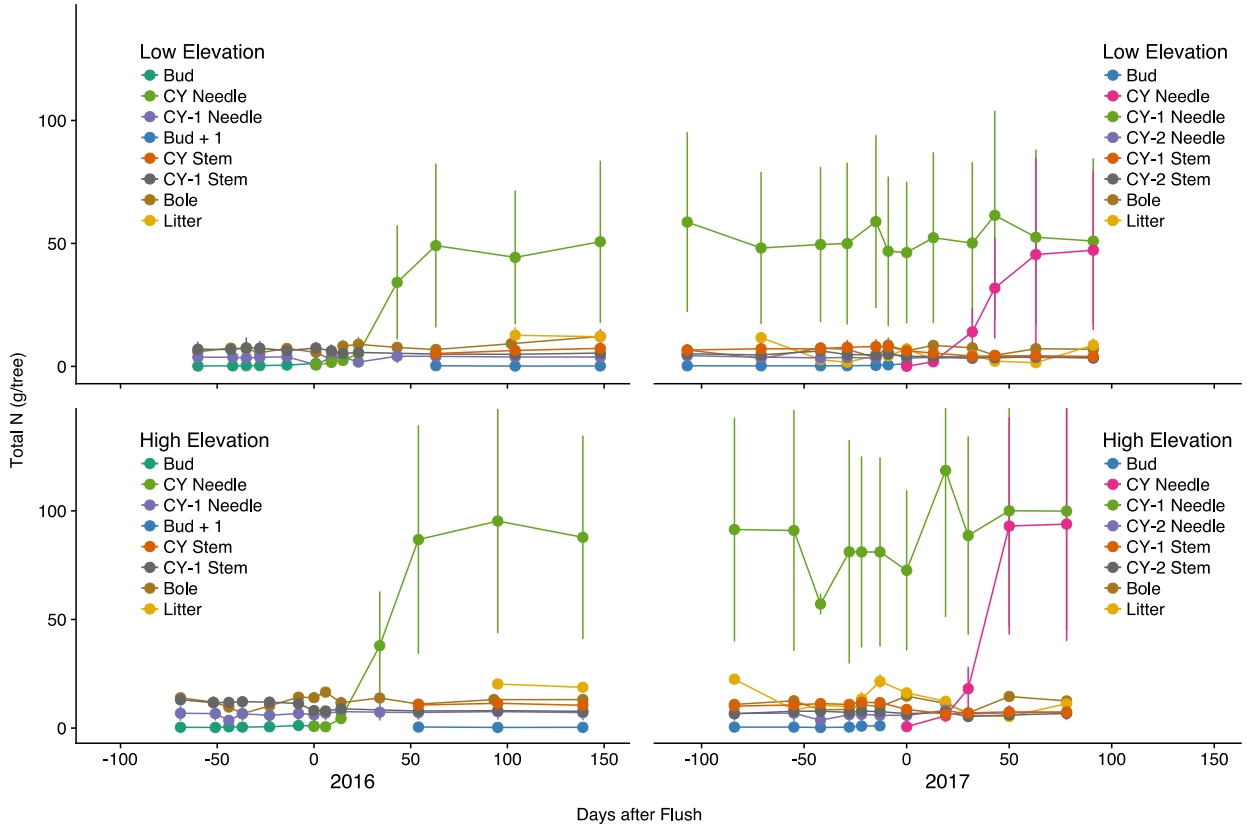

Figure 1.7 - New needles at both the low elevation site (top panel) at and at the high elevation site (bottom panel) accumulated N without drawing down N from older growth fractions. Points are means of 5 samples, and error bars represent the standard errors.

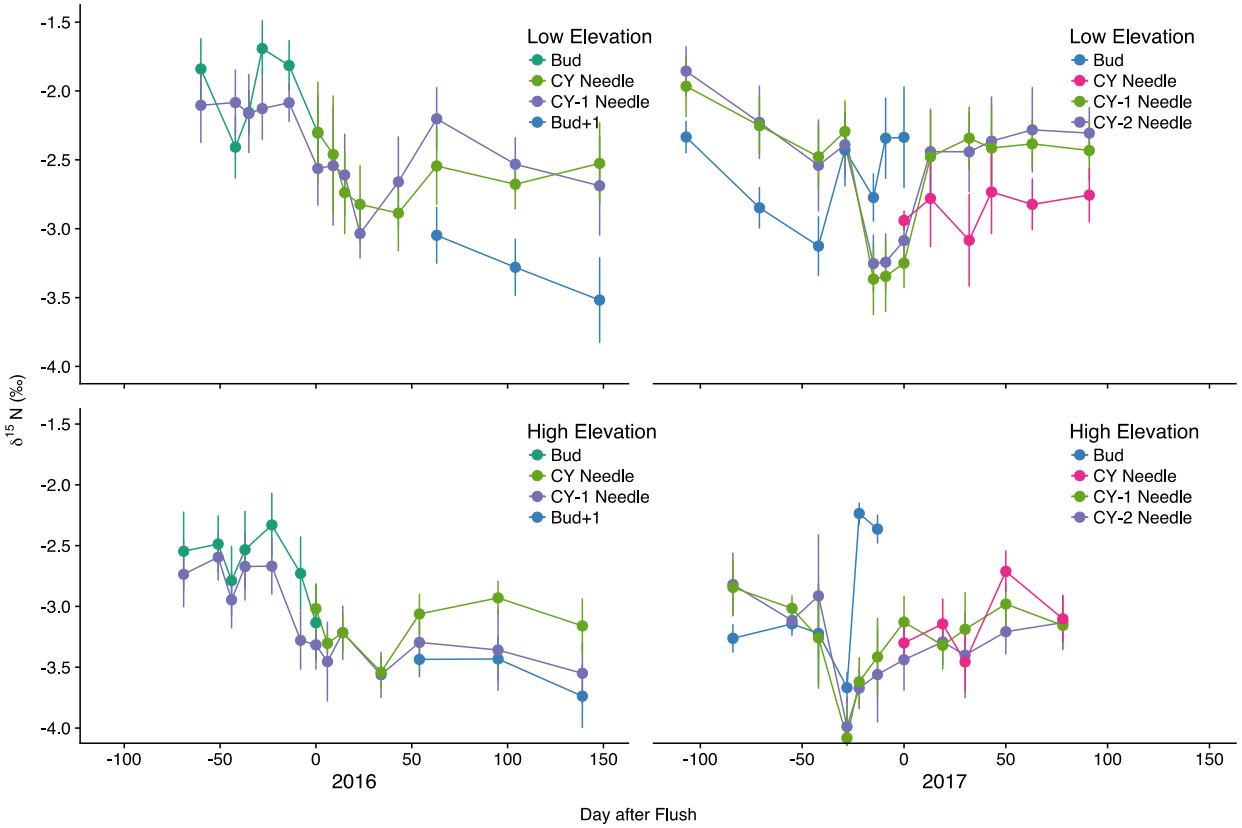

Figure 1.8 - $\delta^{15}N$ in all growth fractions sampled became more depleted around bud break and then more enriched following the depletion event. Points are means of 5 samples, and error bars represent the standard errors.

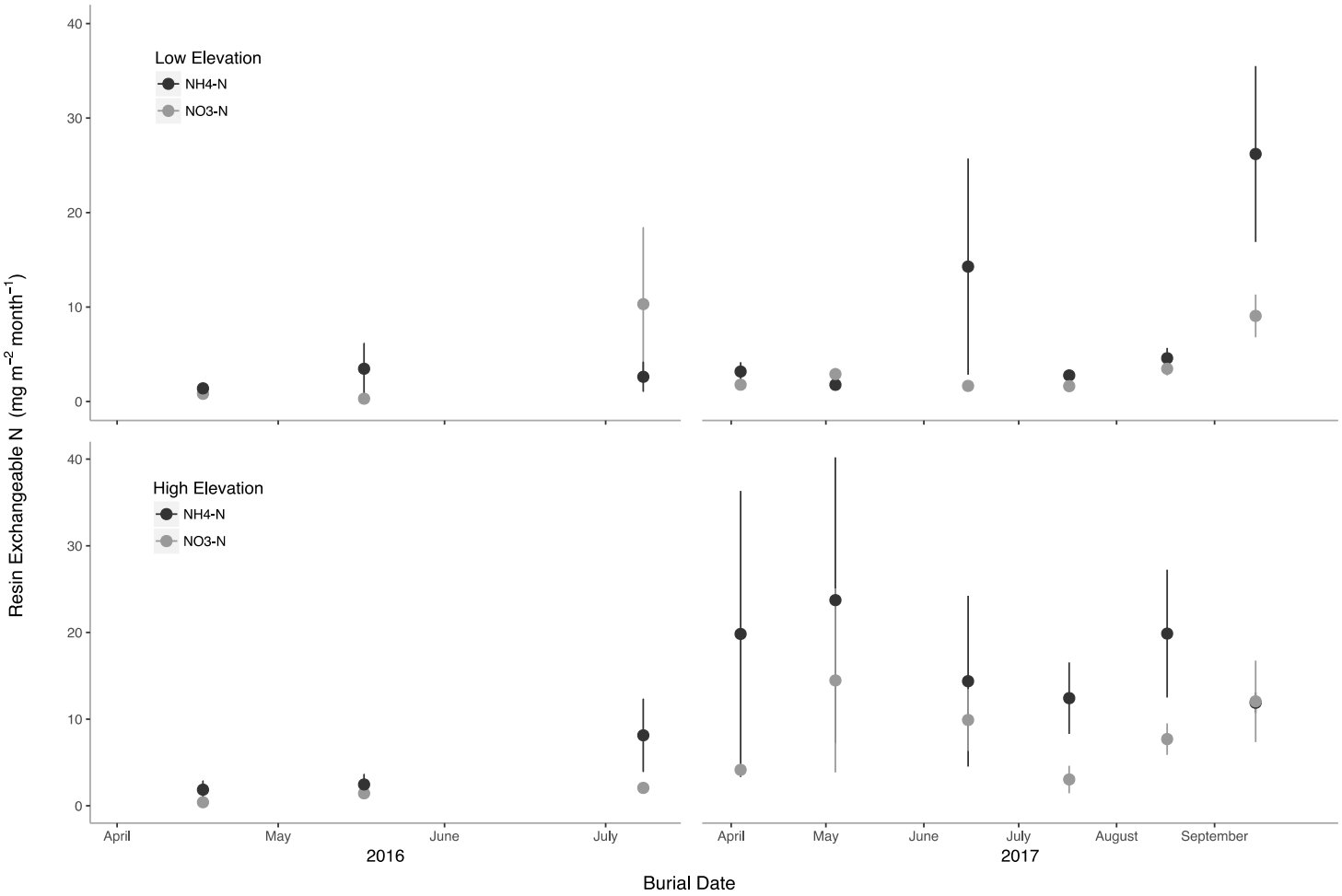

Figure 1.9 – Exchangeable N from the IER probes at the low elevation (top panel) and at the high elevation (bottom panel) was generally low during the early growing season and then increased towards the end of the season. Points represent the means of five samples buried for a month long period, and error bars represent the standard errors.

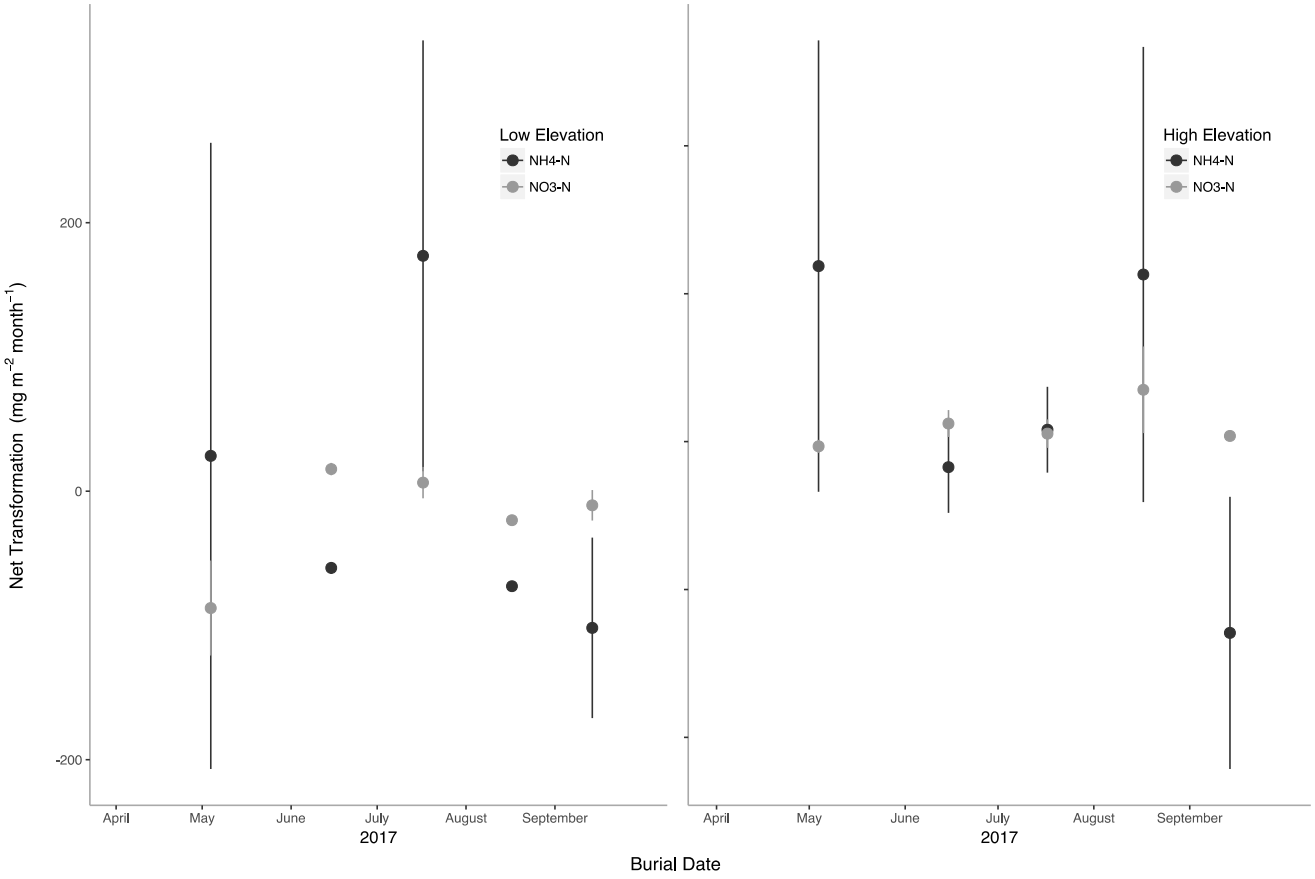

Figure 1.10 – Net transformation rates from the buried bags at the low elevation (left panel) and the high elevation (right panel) showed no statistical differences across the 2017 season. Each point represents the mean of five samples buried for a month long period, and error bars represent the standard errors.

CHAPTER THREE

CLIMATE AND INVASION DRIVE SOIL NUTRIENT DYNAMICS IN TROPICAL MONTANE FORESTS OF THE GALAPAGOS ARCHIPELAGO

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

Author: Claire Qubain

Contributions: CAQ secured funding, developed the sampling protocol, collected samples, performed the chemical and statistical analysis, and wrote the first draft of the manuscript.

Co-Author: Diego Riveros-Iregui

Contributions: DR-I developed the sampling protocol, collected samples, secured permits, and provided feedback on the manuscript.

Co-Author: Jia Hu

Contributions: JH secured permits, developed the sampling protocol, collected samples, and provided feedback on the manuscript.

Manuscript Information

Claire Qubain, Diego Riveros-Iregui, Jia Hu

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Status of Manuscript:
__X__ Prepared for submission to a peer-reviewed journal
_____ Officially submitted to a peer-reviewed journal
_____ Accepted by a peer-reviewed journal
_____ Published in a peer-reviewed journal

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CLIMATE AND INVASION DRIVE SOIL NUTRIENT DYNAMICS IN TROPICAL MONTANE FORESTS OF THE GALAPAGOS ARCHIPELAGO

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Keywords: climate**,** invasion, phosphorus, nitrogen

Abstract

We examined how climate and plant invasion influence soil nitrogen (N) and phosphorus (P) in tropical montane forests on San Cristóbal Island, Galápagos. We collected soils at the end of the warm and wet season and at the end of the cool and dry season along an elevational gradient and under native and non-native plant canopies. The elevation gradient represents a climosequence of temperature, precipitation, relative humidity, and volumetric water content on San Cristóbal. We also analyzed plant tissues for foliar N concentrations to compare native and non-native plant nutrient use. Ammonium (NH_4^+) and phosphate (PO_4^{3-}) increased along the elevational gradient while nitrate (NO_3^-) was most concentrated at the high elevation. Compared to under non-native plant canopies, NH_4^+ was 32% more concentrated under native canopies while NO_3^- was 43% more concentrated under native canopies. We did not detect any differences in PO_4^{3-} pools under native versus non-native plant canopies. Leaching and denitrification likely caused export of NO_3^- during the wet season and at wetter elevations, while rapid turnover rates and plant uptake induced seasonal and elevational differences in PO_4^{3-}. We hypothesized that NH_4^+ was not as variable seasonally because it is not as strongly

controlled by leaching as are NO_3^- and PO_4^{3-}. Overall, understanding nutrient dynamics in the archipelago will allow land managers to decide how to control non-native plants as well as to maintain an understanding of nutrient dynamics in a heavily invaded ecosystem in the future.

Introduction

The processes of soil formation and biogeochemical cycling are driven by five state factors: topography, parent material, time, climate, and potential biota (Jenny 1941). Topography influences nutrient dynamics through leaching and erosion (Weintraub et al. 2014), while different parent materials alter the nutrient composition of overlying soils (Yavitt 2000). Phosphorus is most abundant in young soils, while nitrogen (N) transformation by microbes increases in older soils (Lambers et al. 2008b). Climate influences nutrient dynamics both directly and indirectly through weathering, leaching and erosion as well as through influencing microbial activity and nitrogen transformation (Richardson et al. 2004; Chapin et al. 2011). In addition to the influence of climate, plant community composition can also change biogeochemical cycling under vegetation canopies (Zinke 1962; Sturm et al. 2005). Island ecosystems have served as natural laboratories to conduct studies of pedogenesis because each formation process can be more easily isolated. Islands present strong elevation gradients that drive climate variability across a small area, develop along chronosequences within archipelagos, and host different microbial and vegetation communities across space. While the Hawaiian Islands have served as a central site for many studies of soil formation, other archipelagos, such as the Galápagos, have similar characteristics but have received less

attention. On San Cristóbal Island in the Galápagos Archipelago, climate and biota are two key state factors that influence nutrient dynamics.

Climate patterns in the Galápagos are quite variable. The archipelago experiences two seasons, the cool season (June –December), and the warm season (January – May), due to an inter-annual migration of the Inter-Tropical Convergence Zone. Temperature and precipitation vary along the elevation gradient and between the leeward and windward sides of the islands (Snell and Rea 1999). The climo-sequence is divided between the very arid to arid zone at low elevations, the transition zone, and the humid to very humid zone at high elevations (Percy et al. 2016). Higher elevations are cooler and receive more precipitation in the form of fog (Violette et al. 2014), while lower elevations are dryer and typically only receive rainfall. However, the climate regime in the Galápagos is predicted to change due to intensification of the El Niño Southern Oscillation (ENSO) (Trueman and d'Ozouville 2010). The Galápagos will experience more intense drought in La Niña years followed by prolonged rainfall events during El Niño years. Longer droughts and prolific rainstorms will likely alter rates of parent material weathering and soil development (White and Blum 1995), but we lack thorough knowledge of soil processes on the islands to detect those changes. Therefore, establishing basic knowledge of soil N and P chemistry on the islands will become more important as the climate regime intensifies.

In addition to climate effects, plant invasions influence nutrient cycling in tropical island ecosystems. In general, plant invasion can increase N availability, mineralization rates, and litter decomposition rates (Ehrenfeld 2003). Invasive plants not only directly

affect nutrient cycling, but they also trigger indirect positive feedbacks in soil nutrient dynamics. Added nutrients and accelerated biogeochemical cycling due to introduced species increase the abundance of additional non-natives (Ostertag and Verville 2002). While most research suggests that invasive plants increase soil nutrient availability and decomposition rates, not all areas respond following these patterns; some studies find declines or no change in soil nutrient availability after plant invasion (Ehrenfeld and Scott 2001). Therefore, examining invasion on an individual species or location basis will allow for the detection of potentially unpredictable effects of plant invasion on ecosystem processes. Furthermore, studying these basic nutrient dynamics could influence how land managers implement native plant restoration.

In the Galápagos, plant invasions may alter biogeochemical processes; on one of the older islands, San Cristóbal, 70% of the island is impacted by land use change and plant invasion, and since 1987, 77% of the native vegetation across the islands has been converted to non-native (Villa and Segarra 2010). Many farmers have abandoned their crops to work in the more lucrative tourism industry, and as a result, agricultural species, like guava *(Psidium guajava)* and blackberry (*Rubus niveus*), grow uncontrolled. The non-native vegetation may be influencing nutrient dynamics on San Cristóbal, but their biogeochemical impact has not been thoroughly examined. To date, only a handful of studies have focused on characterizing soil N or P concentrations in the Galápagos. (Kitayama and Itow 1999; Chacón 2010; de la Torre 2013; Jäger et al. 2013). To our knowledge, no studies have examined soil N and P availability across the strong elevation gradient on San Cristóbal. These observations leave many avenues for discovery of

physical and biological controls on soil nutrient dynamics.

The main objective was to characterize seasonal soil N and P concentrations on San Cristóbal, Galápagos. We evaluated how climate controls seasonal nutrient concentrations through the influence of temperature, precipitation and soil moisture, and we characterized how non-native plant species mediated changes in soil N and P concentrations across the island's elevation gradient. Few studies have examined these processes thoroughly in the Galápagos, and further investigation is necessary to inform the management of ecosystem change in the Galápagos.

Methods

Site Description

The Galápagos Archipelago is located 1000 km off the coast of Ecuador and is comprised of 13 main islands. They formed when the Nazca Plate passed over a hot spot in the Pacific Ocean, causing volcanic eruptions, and eventually islands (Geist et al. 2014). Of the islands in the archipelago, this study was performed on San Cristóbal Island, one of the oldest, dated to 2.35 ma (Geist et al. 2014). On the leeward, drier side of the island, we sampled soils along an elevation gradient at low (300 m above sea level (a.s.l.)), mid (500 m a.s.l.) and high (650 m a.s.l.) elevation sites under both native and invasive plant canopies. *Bursera graveolens* (torchwood), *Zanthoxylum fagara* (cat's claw), and *Miconia robinsoniana* (miconia) are dominant native plant species at the low, mid, and high elevations, respectively. However, non-native species, such as *Psidium guajava* (guava) and *Rubus niveus* (blackberry), are dominant at all elevations sampled.

Study sites are managed to varying degrees. The low and high elevation sites are

managed to maintain native species, while the mid elevation site is relatively unmanaged. The low elevation site is a managed farm where in the last 3-4 years, in one plot, non-native species were removed and native species were replanted, and in another plot, non-native species were not removed. In the restored plot at the low elevation, non-native crop plants were planted and manure was applied as fertilizer on the crop plants. At both the mid elevation and high elevation, native and non-native plants are interspersed, but at the high elevation, non-native eradication efforts are taking place and *Miconia ribsoniana* was replanted in the last 5-10 years.

Typical of many tropical islands, the Galápagos experience a warm and wet season (January–May) and a cool and dry season (June–December). From 1977 to 1983 on San Cristóbal at *c.* 6 m.a.s.l, the mean annual temperature was 24.8°C, and the mean temperature during the cool season was 23.5°C and was 26.5°C during the warm season (Violette et al. 2014). The estimated mean annual rainfall on San Cristóbal was 2961 mm/year above 650 m.a.s.l., and the measured mean annual rainfall at *c.* 6m a.s.l was 368 mm/year (Pryet 2011; Violette et al. 2014). Based on evidence from Santa Cruz island, an additional 26% of the median annual rainfall in the highlands occurs in the form of occult precipitation (fog), but fog is rarely present at the low elevations (Pryet et al. 2012).

Meteorological Data

EM50 data loggers recorded air temperature, relative humidity, precipitation, and volumetric water content (VWC) at 15-minute intervals at each of the three sites (METER Group, Pullman, WA). To measure VWC, EC-5 sensors were buried at 10, 20, and 50 cm at the mid and high elevation sites and at 10, 20, and 30 cm at the low

elevation site. Due to sensor malfunctions at the high elevation site, air temperature and relative humidity were not recorded in 2017. However, temperature inside the data logger was recorded. In order to estimate the air temperature at the high elevation site, I modeled outside air temperature as a function the temperature inside the data loggers from both the low and mid elevation sites. I then used that model to predict air temperature at the high elevation site. Further, at the low elevation site, two of the three VWC probes malfunctioned, so only values from the 30 cm depth were reported. The average VWC from all three depths was presented for the mid and high elevation sites.

Soil Sampling

At each of the three sites, we collected 15 samples at a depth of 15cm from under native plant canopies and 15 samples from under non-native plant canopies, for a total of 30 samples per site. Samples were collected in May 2017 at the end of the wet season/start of dry season and in December 2017 at the end of the dry season/start of wet season. Within 24 hours of sample collection, ammonium (NH_4^+) and nitrate (NO_3^-) were extracted from the soil samples in a solution of 2M KCl for one hour. All live plant material and rocks were manually removed from the soil cores. Samples were inverted by hand every 15 minutes across the one-hour extraction period, and then they were gravity filtered with p8 coarse paper filters and syringe filtered through 0.7 µm glass tips. After extraction, samples were stored frozen until analysis. Remaining soil was reserved, transported, and then air-dried for one month. We extracted phosphate (PO_4^{3-}) in a solution of ammonium fluoride and hydrochloric acid following a Type I Bray's extraction procedure. Soil solutions were shaken by hand for five minutes and then

filtered in the same manner as the N extracts. All extracts were frozen for storage and then analyzed using flow injection analysis (Lachat Quik-Chem Series 2400, Colorado).

Plant Sampling

During each sampling period, we collected leaf samples from the vegetation we sampled soils under. All samples were transported back to the lab and dried at 60°C for 48 hours. Leaves were hand ground using liquid nitrogen and then weighed into tin capsules. Then the samples were analyzed for %N and %C using a CHNOS Elemental Analyzer. Due to small sample size, statistical analyses were not conducted. However, more samples will be analyzed in the future.

Statistical Analysis

In order to examine spatial and temporal patterns of NH_4^+, and NO_3, and PO_4^{3-} concentrations, we fit linear mixed effect models using the lme function in R (Pinheiro et al. 2014; R Core Team 2017). To select each model, we used a stepwise AIC process (Table 2). We started with a full model testing a three-way interaction between elevation, plant canopy type, and season, after accounting for the random effect of sampling location. We removed variables or interactions one at a time and selected the model with the lowest AIC score. After visually assessing if each model distribution met the assumptions of normality and constant variance using residual and normal-QQ plots, the concentrations of each nutrient were log transformed.

Results

Soil Sampling

We examined differences in NH_4^+, NO_3^-, and PO_4^{3-} across three elevations (300m, 500m, and 650 m), between the wet and the dry season, and under native and non-native plant canopies. For each analysis, we first fit a full linear mixed effects model including a three-way interaction between elevation, season, and plant type (native or non-native), after accounting for a random effect of sampling location. We then used a stepwise AIC process to select each model (Table 2). For NH_4^+, we selected the following model:

$$\text{Log}(NH_4^+) = ß_0 + ß_1I_{300m} + ß_2I_{500m} + ß_3I_{650m} + ß_4I_w + ß_5I_d + ß_6I_n + ß_7I_{in} + ß_8I_{300m} * I_w + ß_9I_{500m} * I_w + ß_{10}I_{650m} * I_w + ß_{11}I_{300m} * I_d + ß_{12}I_{500m} * I_d + ß_{13}I_{650m} * I_d + ß_{14}I_w * I_n + ß_{15}I_w * I_{in} + ß_{16}I_d * I_n + ß_{17}I_d * I_{in} + \text{location}_i + \varepsilon_{ij}, \text{location}_i \sim N(0, \sigma^2), \varepsilon_{ij} \sim N(0, \sigma^2_\varepsilon),$$

where 300m, 500m, and 650m refer to the site elevation expressed in meters; w and d refer to wet or dry season; n and in refer to native or invasive vegetation, ε_{ij} = residual variability of the j^{th} occasion for the i^{th} subject, and $N(0, \sigma^2)$ denotes that errors are normally distributed with a mean of 0 and a variance of σ^2.

At the mid and high elevation sites, median NH_4^+ was higher during the dry season, but at the low elevation site, median NH_4^+ was higher during the wet season. In the dry season, NH_4^+ increased along the elevation gradient from 5.47 mg Kg^{-1} (95% confidence interval (CI) of 4.48 to 6.68 mg Kg^{-1}) at the low elevation to 11.59 mg Kg^{-1} at the high elevation (95% CI of 9.02 to 14.15 mg Kg^{-1}, respectively). However, during the wet season, NH_4^+ did not vary across the elevations. During the dry season, there was no difference in NH_4^+ concentrations under native or non-native plant canopies. However, during the wet season, NH_4^+ concentrations were 2.69 mg Kg^{-1} greater under native versus non-native plant canopies (95% CI of 2.54 to 3.28). We also found an interaction

between elevation and season (χ^2 (2,81 df) =3.70, p-value = 0.03), suggesting that elevational differences in median NH_4^+ concentrations depended on the season sampled. Furthermore, we detected evidence for an interaction between NH_4^+ concentrations and vegetation type (χ^2 (1,81 df) = 4.86, p-value = 0.03), suggesting differences in soil median NH_4^+ concentrations between plant types also depended on the season sampled.

To examine differences in NO_3^- concentrations between elevations, seasons, and plant type, we selected the following model:

$$\text{Log}(NO_3^-) = ß_0 + ß_1 I_{300m} + ß_2 I_{500m} + ß_3 I_{650m} + ß_4 I_w + ß_5 I_d + ß_6 I_n + ß_7 I_{in} + ß_8 I_{300m} * I_w + ß_9 I_{500m} * I_w + ß_{10} I_{650m} * I_w + ß_{11} I_{300m} * I_d + ß_{12} I_{500m} * I_d + ß_{13} I_{650m} * I_d + \text{location}_i + \varepsilon_{ij},$$
$$\text{location}_i \sim N(0, \sigma^2), \varepsilon_{ij} \sim N(0, \sigma^2_\varepsilon)$$

During the dry season, NO_3^- did not vary across elevations and ranged from 4.9 mg Kg^{-1} at the low elevation (95% CI of 4.05 to 6.04 mg Kg^{-1}) to 6.0 mg Kg^{-1} at the high elevation (95% CI 4.95 to 8.17 mg Kg^{-1}). However, during the wet season, the median NO_3^- concentration at the low elevation was 6.04 mg Kg^{-1} (95% CI of 4.95 to 7.39 mg Kg^{-1}), which was 17 times greater than at the high elevation and 35 times greater than that at the mid-elevation. The median NO_3^- concentration under native plant canopies was 0.59 mg Kg^{-1} greater than under non-native plant canopies (χ2 (1,86 df) =32.10, p-value < 0.0001). We detected an interaction between NO3- and elevation, suggesting that differences in median NO3- concentrations between elevations depended on the season sampled (χ2 (2, 82 df) = 103.06, p-value < 0.0001).

We selected the following model to examine differences in PO_4^{3-} concentrations between elevations and seasons:

$Log(PO_4^{3-}) = ß_0 + ß_1I_{300m} + ß_2I_{500m} + ß_3I_{650m} + ß_4I_w + ß_5I_d + ß_6I_{300m} * I_w + ß_7I_{500m} * I_w + ß_8I_{650m} * I_w + ß_9I_{300m} * I_d + ß_{10}I_{500m} * I_d + ß_{11}I_{650m} * I_d + location_i + \varepsilon_{ij}$, $location_i \sim N(0, \sigma^2)$, $\varepsilon_{ij} \sim N(0, \sigma^2\varepsilon)$

For PO_4^{3-}, we found that plant type did not influence PO_4^{3-} concentrations (p-value > 0.05), so it was not included in the final model. We still detected that the elevational differences in median PO_4^{3-} concentrations depended on the season sampled (χ^2 (2, 87 df) = 4.57, p-value= 0.01). During both seasons, PO_4^{3-} was most concentrated at the high elevation site but did not differ between the low and mid-elevations. The median PO_4^{3-} concentration at the high elevation during the wet season was 51.93 mg Kg^{-1} (95% CI of 40.44 to 66.69 mg Kg^{-1}) and was 134.30 mg Kg^{-1} during the dry season (95% CI of 99.48 to 148.41 mg Kg^{-1}). The seasonal differences at the low and mid-elevations each differed by less than three mg Kg^{-1}.

Foliar N in natives versus non-natives

Preliminary examinations showed that foliar %N was higher in native compared to non-native species at two of the three elevations sampled (Table 1). At the low elevation, the mean foliar %N of three native species, *Bursera graveolens, Chiocacca alba,* and *Leocarpus darwinii,* was 0.35% greater than that of the non-native species *Psidium guayaba*. At the mid elevation, the foliar %N in the native species *Zanthoxylum fagara* was 1.0% greater than in the mean %N of two non-native individuals, *Psidium guayaba* and *Pennisetum purpureum*. At the high elevation, when five samples from the dominant native species, *Miconia ribsoniana*, and the dominant non-native species, *Psidium guayaba*, were compared, the mean %N was 0.55% greater in the non-native.

Discussion

Seasonal and elevational changes in climate and the presence of non-native plants influenced soil N and P pools in the tropical montane forests on San Cristóbal Island, Galápagos. Soil N and P varied between elevations but typically depended on the season sampled, with precipitation, temperature, and soil moisture influencing soil nutrient dynamics (Jenny 1941; Vitousek and Chadwick 2013). On the other hand, the influence of non-native plants on soil N and P pools in this study did not agree with the general consensus that nutrient pools increase under non-native canopies (Liao et al. 2008). Instead, we discovered that soil N pools decreased under non-native canopies while available P did not change between the two plant types.

Nitrogen Pools

Climate controlled nutrient dynamics on San Cristóbal Island, but the effect sizes depended on the season sampled. This result is expected, since rainfall and temperature gradients also determine thresholds of soil development in other ecosystems (Ewing et al.; Chadwick et al. 2003; Vitousek and Chadwick 2013). Here, NH_4^+ increased with elevation in the dry season but did not vary with elevation during the wet season. NO_3^- concentrations decreased with elevation during the dry season. During the wet season, NO_3^- was most concentrated at the low elevation and least concentrated at the mid elevation. Leaching and denitrification likely caused export of NO_3^-, a highly mobile ion, out of the system during the wet season and at wetter elevations (Chapin III et al. 2011; Weintraub et al. 2014), but NH_4^+ was less variable across the elevation gradient and between seasons, presumably because it is not as prone to leaching and denitrification as

NO3-. Interestingly, Chacón (2010) observed that both NH_4^+ and NO_3^-, not just NH_4^+, increased along the elevation gradient on neighboring Santa Cruz Island. However, N concentrations in our study still fell within the N concentration ranges observed in other soils in the Galápagos (Kitayama and Itow 1999; Chacón 2010; Jäger et al. 2013). The differences in N pools across elevation found between this study and others could have been due to the age of the islands where observations were made, to the sampling season, or to varying levels of disturbance or land use where sampling took place. Some of the studies focused their sampling on one island zone or on one season. Further, sampling could have occurred in sites with greater or lesser disturbance or land use change, but there is little way to compare between studies without knowing specific sites.

We detected that NH_4^+ and NO_3^- were more concentrated under native than under non-native canopies. However, most studies examining how invasion influences nutrient dynamics find that soil nutrient pools are more concentrated under non-native canopies (Vitousek et al. 1987; Scott et al. 2001; Ehrenfeld 2003, 2010; Liao et al. 2008; Vilà et al. 2011). Because the magnitude and direction of changes in nutrient pools in invaded systems varies greatly depending on plant functional traits, ecosystem types, and N-fixation status of invaders, Liao et al. performed a meta-analysis to generalize how invasion influences ecosystem properties. Based on 94 studies, they determined that NH_4^+ and NO_3^- pools under non-native plant canopies were 30% and 17% greater, respectively, compared to under native plant canopies (Liao et al. 2008). In this study, however, NH_4^+ and NO_3^- concentrations were 33% and 42% lower, respectively, under non-native canopies than native canopies.

In other studies conducted in the Galápagos, non-native plants typically increased soil N pools. In the arid zone on San Cristóbal Island, percent N (%N) in bulk soil was highest in a pasture restored with native species and lowest in actively managed agricultural lands containing non-natives (de la Torre 2013). In the humid zone on Santa Cruz, NH_4^+ was more concentrated under non-native canopies, but they detected no differences in NO_3^- under different canopy types (Jäger et al. 2013). Whereas we examined numerous non-native and native species growing in all climate zones on San Cristóbal, other studies carried out on the Galápagos either followed the influence of a single non-native species or of plant communities in single climatic zones, which may have influenced the magnitude of non-natives' influence on soil chemistry. Increases in soil N pools after invasion are far from universal, however, and decreases or no differences, as we saw in this study, were also observed (Ehrenfeld and Scott 2001; Svejcar and Sheley 2001; Martin et al. 2009; Scharfy et al. 2009; Ehrenfeld 2010).

Phosphorus Pools

Climate was the strongest control of PO_4^{3-} dynamics in the Galápagos. PO_4^{3-} concentrations were significantly higher at the high elevation site, suggesting that greater soil moisture at the high elevation could have increased the organic P turnover rates, in turn elevating the P pool (Turner et al. 2011). However, this is opposite the result that Chacón (2010) observed on Santa Cruz, where PO_4^{3-} concentrations decreased with increasing elevation. PO_4^{3-} varied seasonally as well, suggesting that plant uptake controlled P pools along shorter time intervals. After the cool, dry season, PO_4^{3-} was

almost double the concentration of PO_4^{3-} after the warm, wet season. Under drier conditions, diffusion of PO_4^{3-} from the soil matrix to root depletion shells slow (Gahoonia et al. 1994), and this process could have caused the build up of PO_4^{3-} during the cooler, drier season. Further, cooler temperatures may have caused plant activity, including nutrient uptake, to decrease, leaving more P available in the soil pool (Lambers et al. 2008a).

We did not observe differences in PO_4^{3-} concentrations in soils under native and non-native plant canopies. Kueffer et al. found a similar result in the Seychelles Islands, where three non-native trees did not induce changes in total soil P under their canopies (Kueffer et al. 2008). However, even though soil nutrient pools may not change, non-natives may still impact litter concentrations or decomposition rates. For example, total P increased in litter mass from *Hieracium pilosella*, a non-native forb growing in New Zealand (Scott 2001), and litter P and litter decomposition rates increased in invaded plots in Hawaii (Allison and Vitousek 2004). Though we did not detect changes in soil P pools, changes in plant litter or alteration of decomposition rates may still be apparent if we were to observe those processes.

Why are non-natives successful in the Galápagos?

Even though non-native species do not benefit from increasing soil nutrient pools on the island, they are still more successful than native plants. Both soil and plant processes observed here suggest that escaping physiological constraints is not what allows non-natives to succeed in this ecosystem. Instead, non-native plants have escaped dispersal limitations (Belyea and Lancaster 1999). In the 1980s, the feral goat population

on the islands grew to around 100,000 individuals, and as a result, rapid plant community changes took place (Coblentz 1978). Goats acted as seed vectors after eating guava and blackberry fruits, and the plants were widely dispersed. Non-native do not escape nutrient limitation in this system, yet they are ubiquitous and abundant because they have escaped dispersal limitations.

Implications

While it is important for land management efforts in the Galápagos to understand soil nutrient dynamics, observations of soil dynamics or vegetation communities are executed under a shifted baseline because the islands are so heavily influenced by invasion (Villa and Segarra 2010; Bush et al. 2014). The Galápagos National Park and some private landowners have made strong efforts to restore native plant species in the last 10 years. While the separate entities are managing land for different practices, such as agriculture or tourism, they have the same goal of mitigating non-natives' impact. This may have influenced the results we found here through legacy effects. At two of our three field sites, land managers and owners had been trying to eradicate non-native species, and restoration efforts are succeeding to varying degrees. Non-native plants could have legacy effects in the soil even after they are eradicated (Jäger et al. 2013; Abraha et al. 2018), or the presence of non-native understory species that had not been controlled may have influenced our findings. Instead of testing soils under native and non-native plants that are sometimes interspersed, native and non-native treatment plots could be more heavily maintained for longer periods to test the influence of non-native plants on soil processes in a more coordinated manner.

Nonetheless, these findings, along with the work of Chacón (2010), present the most complete synopsis of nutrient dynamics in the Galápagos to date. More work is needed to illucidate patterns of soil development along the archipelago's chronosequence, as was performed in the Hawaiian archipelago (Vitousek et al. 1983, 1995; Crews et al. 1995). Further, the Galápagos presents a unique dichotomy between human and natural systems which should be explored in order for conservation efforts to be effective in the future. Overall, understanding nutrient dynamics in the archipelago will allow land managers to decide how to control non-native plants as well as maintain an understanding of nutrient dynamics under a shifted baseline for the future.

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Tables

Site Name	Site Elevation	Soil Type*	Soil Texture*	Mean Soil pH*	Native foliar %N	Non-native foliar %N
Mirador	300 m	Ultisol	Sandy loam, Sandy clay loam, and Clay	6.06	2.22%	1.87%
Cerro Alto	500 m	Ultisol	Loamy sand and Clay	6.02	3.28%	2.16%
El Junco	650 m	Oxisol	Sandy loam	4.05	1.59%	2.14%

Table 2.1 – Site Descriptors - metrics with a * are described by Percy et al., In Prep.

Analyte	Fixed effects	AIC
NH_4^+	Elevation * Season * Plant Type	355.5612
	Elevation*Season + Plant Type *Season	345.837*
	Elevation * Season + Plant Type	346.826
	Elevation + Season + Plant Type	346.808
	Elevation * Season	346.758
	Elevation + Season	346.755
NO_3^-	Elevation * Season * Plant Type	375.051
	Elevation * Season + Plant Type *Season	370.6681
	Elevation * Season + Plant Type	367.063*
	Elevation + Season + Plant Type	483.803
	Elevation * Season	390.261
	Elevation + Season	497.262
PO_4^{3-}	Elevation * Season * Plant Type	390.238
	Elevation * Season + Plant Type *Season	387.382
	Elevation * Season + Plant Type	387.164
	Elevation * Season	382.595*
	Elevation + Season	384.67

Table 2.2 – Results from the stepwise AIC model selection processes. We selected the models with AIC scores listed with an *

Figures

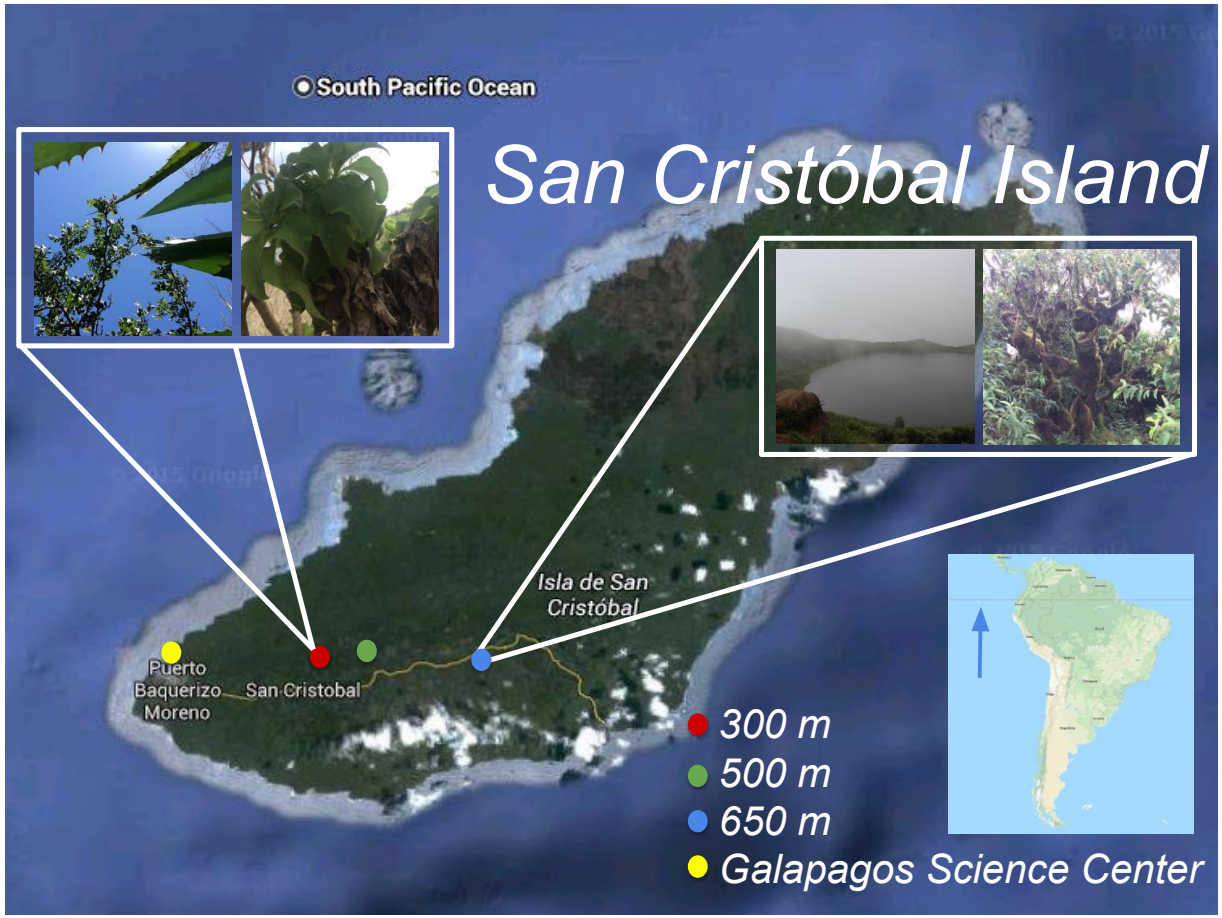

Figure 2.1 – Map of study sites along the elevation gradient with photos demonstrating the difference in vegetation communities along the climosequence. *Zanthoxylum fagara* (left) and *Scalesia gordilloi (right)* pictured at the 300m site, and El Junco lake (l) and *Psidium guyaba* (r) at the high elevation site. Arrow on map inset shows location of the Galápagos Archipelago.

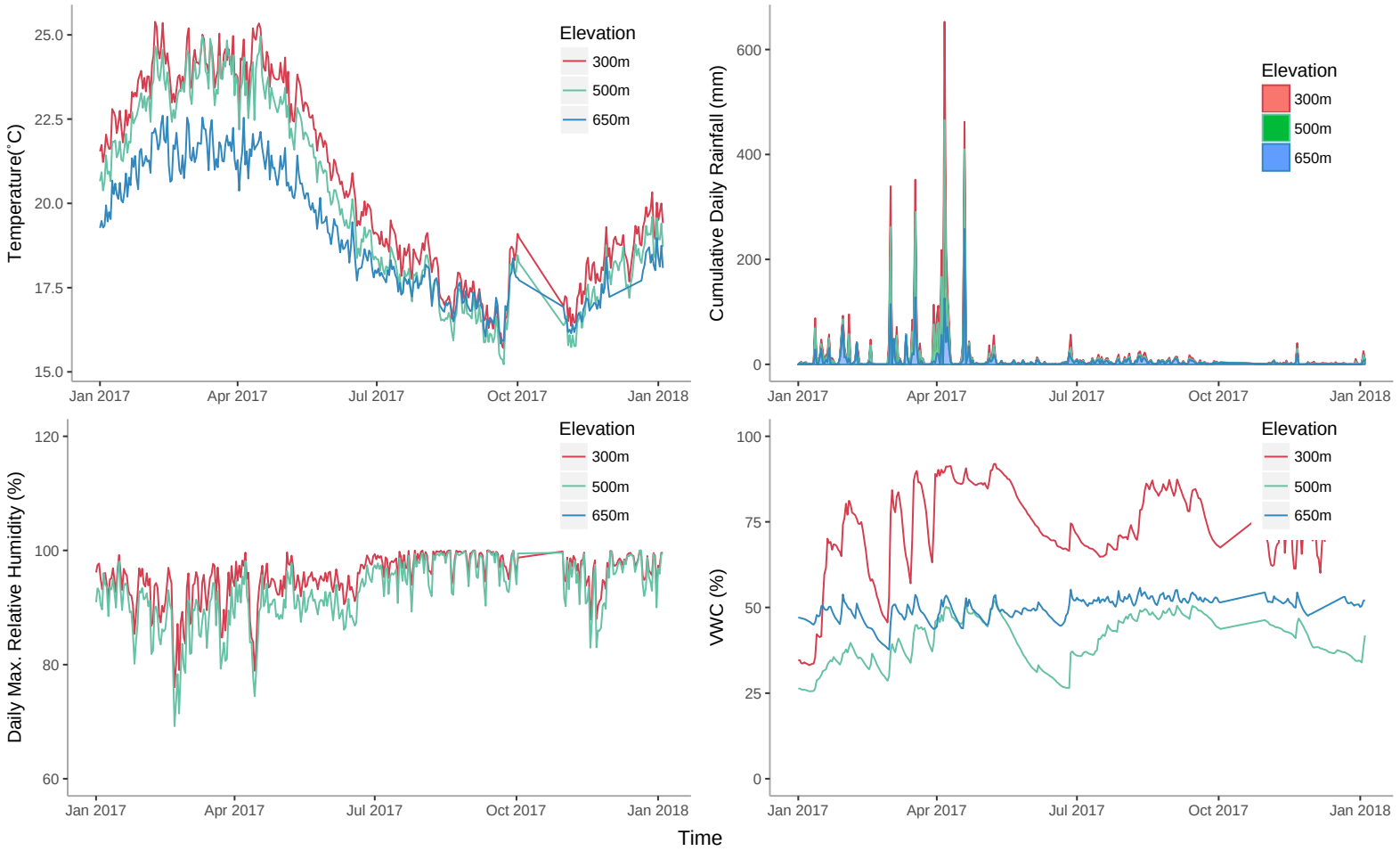

Figure 2.2 - Meteorological measurements of (clockwise) mean daily air temperature, cumulative daily rainfall, VWC, and mean daily maximum RH. Note that the air temperature at the 650 m elevation site is modeled from the temperature inside the data logger, and the VWC at the 300 m elevation site is from the 30 cm depth while the VWC from the 500 m and 650 m elevation sites are from the 20 cm depth.

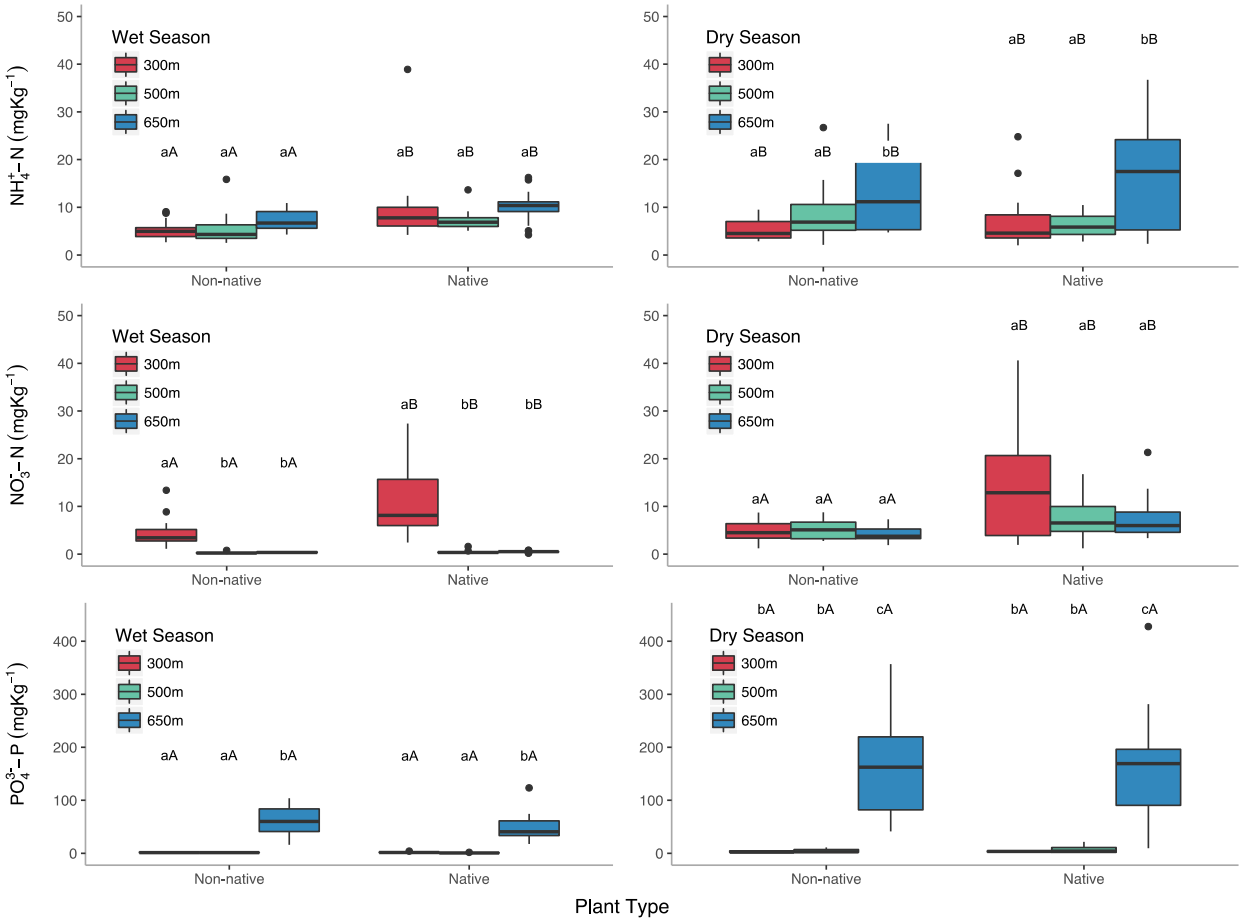

Figure 2.3 - NH_4^+, NO_3^-, and PO_4^{3-} concentrations across elevations and between native or non-native plant canopies sampled under with measurements from the warm season in the left column and values from the cool season in the right column. Lower cases letters denote differences in nutrient pools between elevation and between season and upper case letter denote differences between pools under native and non-native canopies.

CHAPTER FOUR

CONCLUSIONS AND FUTURE WORK

I built on our fundamental understanding of ecosystem function by examining how climate variability influences feedbacks between plant processes and soil nutrient dynamics. At Lubrecht Experimental Forest, I examined how variability in snow depth, precipitation, and soil moisture influenced seasonal nitrogen allocation in Douglas-fir. I then examined if N cycling within Douglas-fir synchronized with patterns of N availability in the soil. In this case, N availability in the soil influenced plant nutrient dynamics. On the other hand, on San Cristóbal Island in the Galápagos Archipelago, plants fed back and influenced soil nutrient dynamics. Changes in precipitation, soil moisture, and temperature strongly controlled nutrient concentrations in the soil, and to a lesser degree, plant community type determined nutrient concentrations, especially N concentrations, in the soil.

While these projects elucidated patterns of basic ecosystem function, further work could augment the findings' impact. For example, while we know that Douglas-fir trees must be accessing most of their N from the soil, it is still uncertain if they are actually using that N to photosynthesize. Answering this question would allow us to conclude if evergreens are N limited or not by testing the hypotheses drawn by Warren and Adams (2004). To examine N-limitation in Douglas-fir, I would measure metabolically active and metabolically inactive Rubisco content and then relate those measurements to trees'

photosynthetic rates across a season. This would allow me to determine if trees are actually using the N they are taking up from the soil across the season.

In the Galápagos, further work is needed to inform restoration of the landscape. However, the restoration must be carried out in a socially minded manner. Residents value some invasive species, like orange trees and guava, for their products, and native species restoration should be carried out in concert with agricultural production. Not all land, though, can be used for production, and the Galapagos National Park must direct and implement most of the restoration efforts. Scientists must guide land management in order for restoration efforts to become effective, and results presented here can inform land managers of impacts that their restoration will have going forward.

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